CAPTISOL™: SBE7-β-Cyclodextrin

A New Drug Formulation System

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Purpose:

CyDex L. C. was established to license and commercialize modified cyclodextrins for use in drug development and formulation. The series of anionically charged sulfobutylether cyclodextrins (SBE-CDs) were originally synthesized and patented by scientists from the University of Kansas Higuchi Biosciences Center for Drug Delivery Research. CyDex has an exclusive license to the SBE-CDs. Two SBE-CD products are commercially available, Captisol™ and Advasep. Captisol™ is exclusively marketed by CyDex for use in drug development and Advasep is marketed as a separation enhancer for use in capillary electrophoresis, particularly for chiral separations. CyDex works with domestic and international companies interested in licensing Captisol™ and other SBE-CD technology. A complimentary sample of Captisol™ is available upon the execution of a confidential disclosure agreement and additional supplies can be purchased.

Management:

The CyDex management team consists of Mr. Peter Higuchi, President and founder of the company; Mr. Edward W. Mehrer, Chief Financial Officer; Mr. Karl W. Strohmeier, VP of Corporate Development, Dr. Diane Thompson, VP of Research and Development; and Ms. Nancy Thrutchley, Director of Operations and Regulatory Affairs.

Mr. Higuchi was previously associated with Marion Merrell Dow in finance, licensing and corporate development. Both Mr. Mehrer, Mr. Strohmeier and Ms. Thrutchley were associated for many years at Marion Labs, Marion Merrell Dow and Hoechst Marion Roussel. Mr. Mehrer was formerly the Chief Financial Officer of Marion Merrell Dow. Mr. Strohmeier's activities span the finance and corporated developments activities and Ms. Thrutchley has over twelve years of experience in regulatory affairs and project management. Dr. Thompson directed the development of the SBE-CDs at the Higuchi Bioscience Center for Drug Delivery Research at the University of Kansas.

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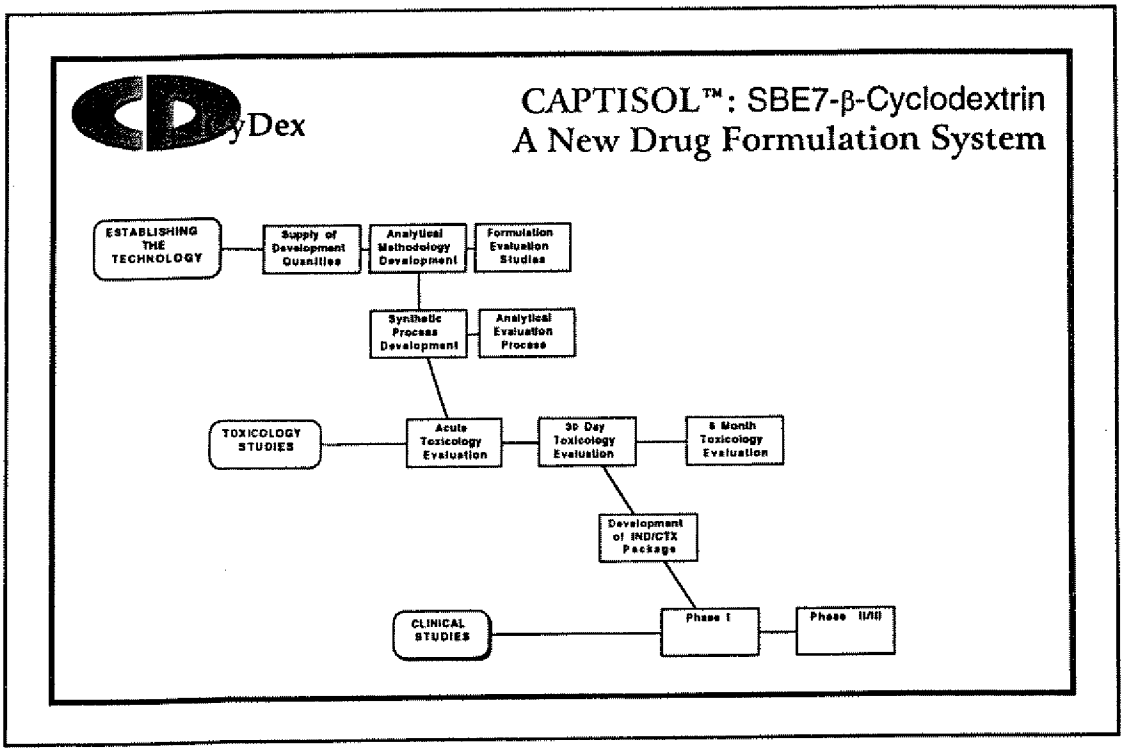
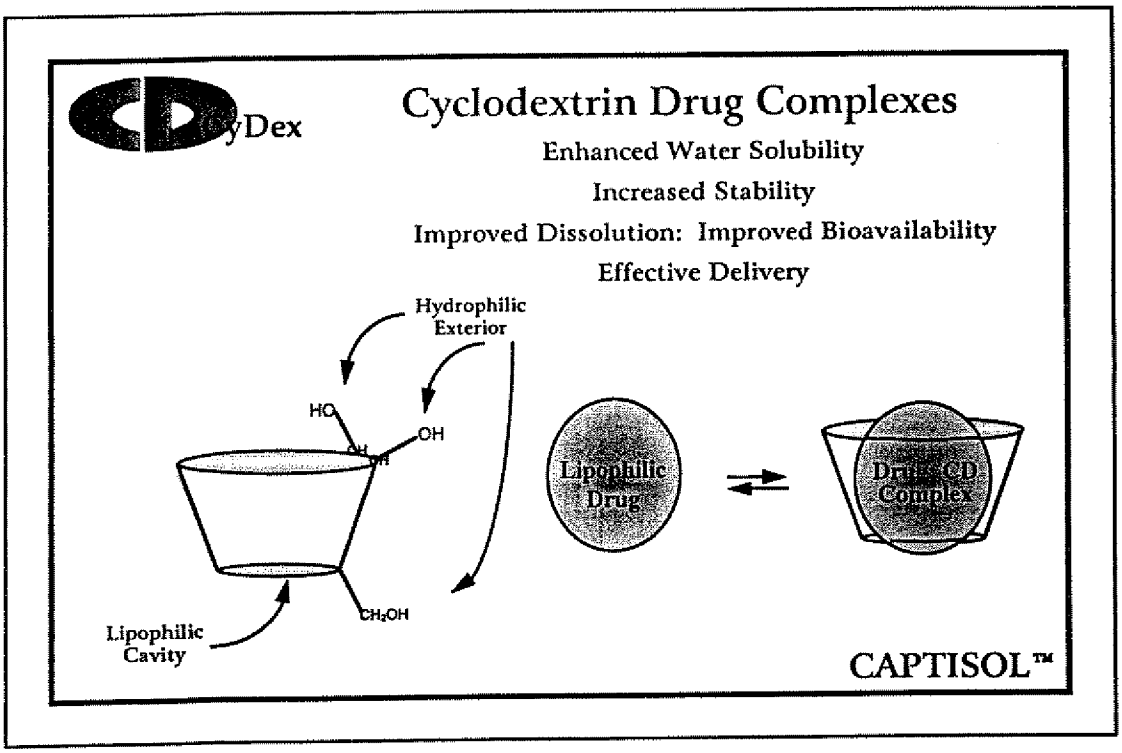


Captisol Development

A series of anionically charged sulfobutylether cyclodextrins (SBE-CDs) were originally synthesized and patented by scientists from the University of Kansas Higuchi Biosciences Center for Drug Delivery Research. CyDex has an exclusive license to the SBE-CDs. Two SBE-CD products are commercially available, Captisol™ and Advasep. Captisol™ is exclusively marketed by CyDex for use in drug development and Advasep is marketed as a separation enhancer for use in capillary electrophoresis, particularly for chiral separations.

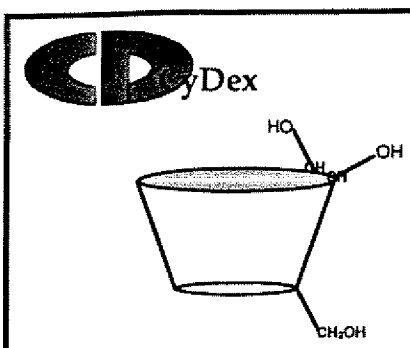
The optimal anionic CD derivative to develop as Captisol was chosen based on determining the effect of structure on complexation, solubility, pharmacological inactivity and systemic safety concerns. The optimal derivative, is the hepta-substituted sulfobutylether derivative of beta-cyclodextrin (SBE7-β-CD = Captisol). This material is devoid of pharmacological activity and exhibits little of the membrane damaging effects of the parent cyclodextrin. The material is highly water soluble and very useful in complexing hydrophobic drug. A quality manufacturing process has been established and control parameters have been determined. Extensive analytical characterization assures a high quality product.

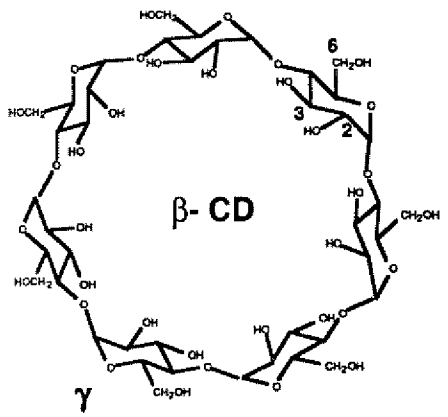
The preclinical animal studies are complete and Captisol has been successfully through Phase I human clinical trials. Therapeutic drug formulations using Captisol are currently in Phase II/III human clinical trials. The results of the completed studies and the plan for future studies are described in the next few slides.



The 3-dimensional structure of the cyclodextrin provides a cavity that is hydrophobic relative to an aqueous environment. The sequestration of hydrophobic drugs inside the cavity of the cyclodextrin can improve:

1) the drug's solubility and stability in water,
2) the rate and extent of dissolution of the lyophilized drug:CD complex, and
3) the bioavailability of the drug when its dissolution and solubility are limiting delivery.





Parent Cyclodextrins	α	β	γ
Glucose Units	6	7	8
Molecular Weight	973	1135	1297
Water solubility (gms/100mL)	12.7	1.88	25.6
Cavity Diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
Cavity Volume (Å)³	~174	~262	~472

CAPTISOL™

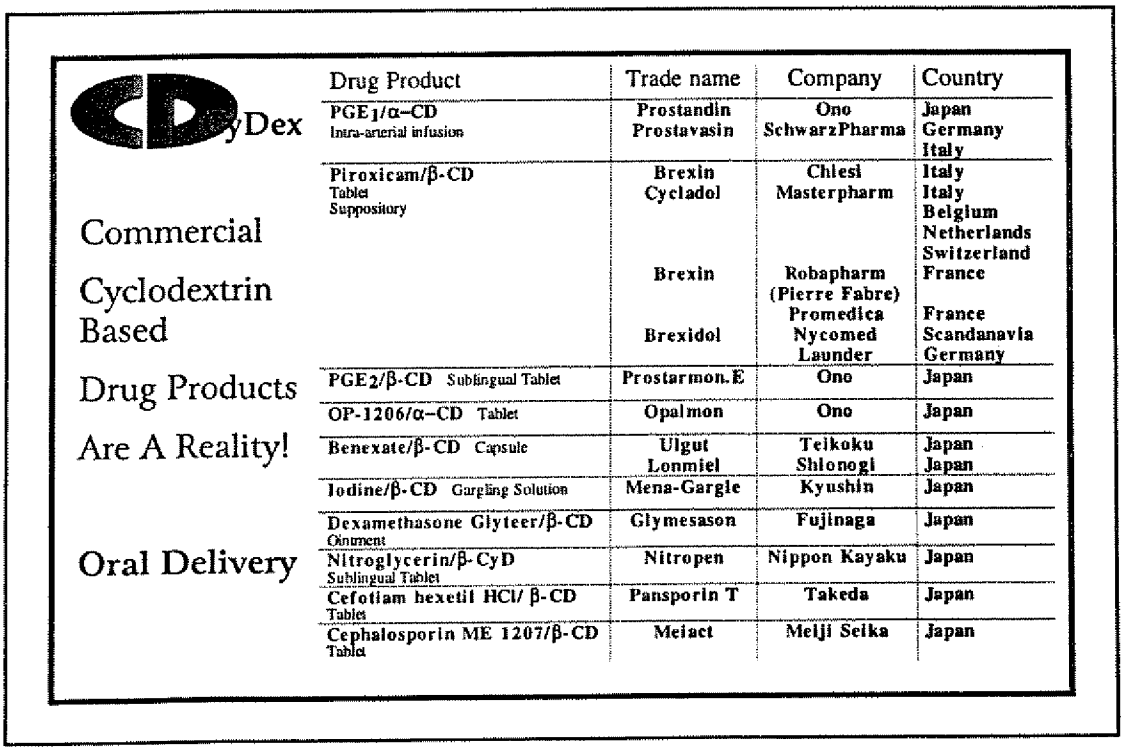
Cyclodextrins have been commercially available since the early 1970's. Three 'parent cyclodextrins' can be produced from the enzymatic conversion of starch. The parent CDs contain 6, 7 or 8 glucopyranose units and are referred to as alpha (α-), beta (β-) and gamma (γ-) cyclodextrin.

The 3D structure of the cyclodextrins can be represented as a segment of a hollow cone. This structure provides a cavity that is hydrophobic relative to the aqueous environment but an exterior surface that is hydrophilic allowing the cyclodextrins to have reasonable water solubilities. Each sugar unit has three hydroxyl substituents that can be chemically derivatized.

Until recently only the β-CD was available in bulk quantities. Luckily the cavity size of this CD accommodates many of the hydrophobic organic units found in traditional small molecule therapeutics. Unfortunately, β-CD has the lowest intrinsic water solubility of the three parent CDs.

β-CD exhibits only a 2% (wt/vol) solubility but this has been sufficient for the successful solubilization and stabilization of a number of drug products. α- and β-CD are utilized in eight commercial therapeutic products in Japan, one in Europe and Japan and one in Europe alone.

These β-CD products are only for oral delivery because β-CD exhibits renal toxicity upon systemic administration. The proposed mechanism of β-CD toxicity involves precipitation of the β-CD or a β-CD:cholesterol complex inside the renal tubule cell. The precipitation is thought to occur due to the low aqueous solubility of β-CD and/or the β-CD cholesterol complex.



Drug Product	Trade name	Company	Country
PGE1/α-CD Intra-arterial infusion	Prostandin Prostavasin	Ono SchwarzPharma	Japan Germany Italy
Piroxicam/β-CD Tablet Suppository	Brexin Cycladol	Chiesi Masterpharm	Italy Italy Belgium Netherlands Switzerland
	Brexin	Robapharm (Pierre Fabre)	France
	Brexidol	Promedica Nycomed Launder	France Scandanavia Germany
PGE2/β-CD Sublingual Tablet	Prostarmon.E	Ono	Japan
OP-1206/α-CD Tablet	Opalmon	Ono	Japan
Benexate/β-CD Capsule	Ulgut Lonmiel	Teikoku Shionogi	Japan Japan
Iodine/β-CD Gargling Solution	Mena-Gargle	Kyushin	Japan
Dexamethasone Glyteer/β-CD Ointment	Glymesason	Fujinaga	Japan
Nitroglycerin/β-CyD Sublingual Tablet	Nitropen	Nippon Kayaku	Japan
Cefotiam hexetil HCl/ β-CD Tablet	Pansporin T	Takeda	Japan
Cephalosporin ME 1207/β-CD Tablet	Meiact	Meiji Seika	Japan

Oral Formulations & Parent Cyclodextrins

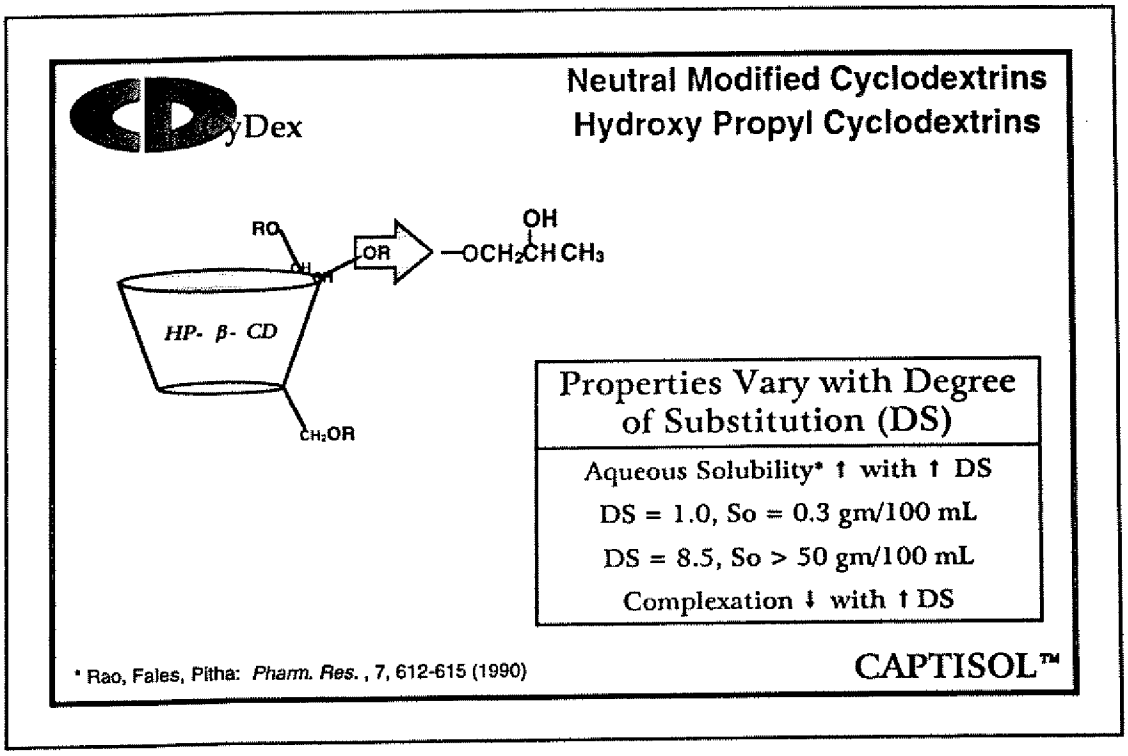
The commercial viability of a cyclodextrin based drug formulation has been established with the marketing of ten products. Eight products have been introduced in Japan, one in Japan and Europe and one in Europe alone. Numerous clinical trials using CD formulations have been conducted or are in progress in the United States, although currently no cyclodextrin-based formulations have been approved.

The faster acceptance of parent cyclodextrin based formulations in Japan and Europe resides primarily in the fact that the parent cyclodextrins have been classified as 'natural starches' and allowed in food products. The extensive use as food additives has provided a historical safety database for oral use.

In the United States, the parent cyclodextrins have not been classified as natural starches but cyclodextrin producers have submitted food additive GRAS petitions for the use of β-cyclodextrins in foods (August 14, 1996). The ultimate approval of β-cyclodextrin as a food additive will increase the use of this material in oral drug formulations in the US.

Parenteral Formulations & Parent Cyclodextrins

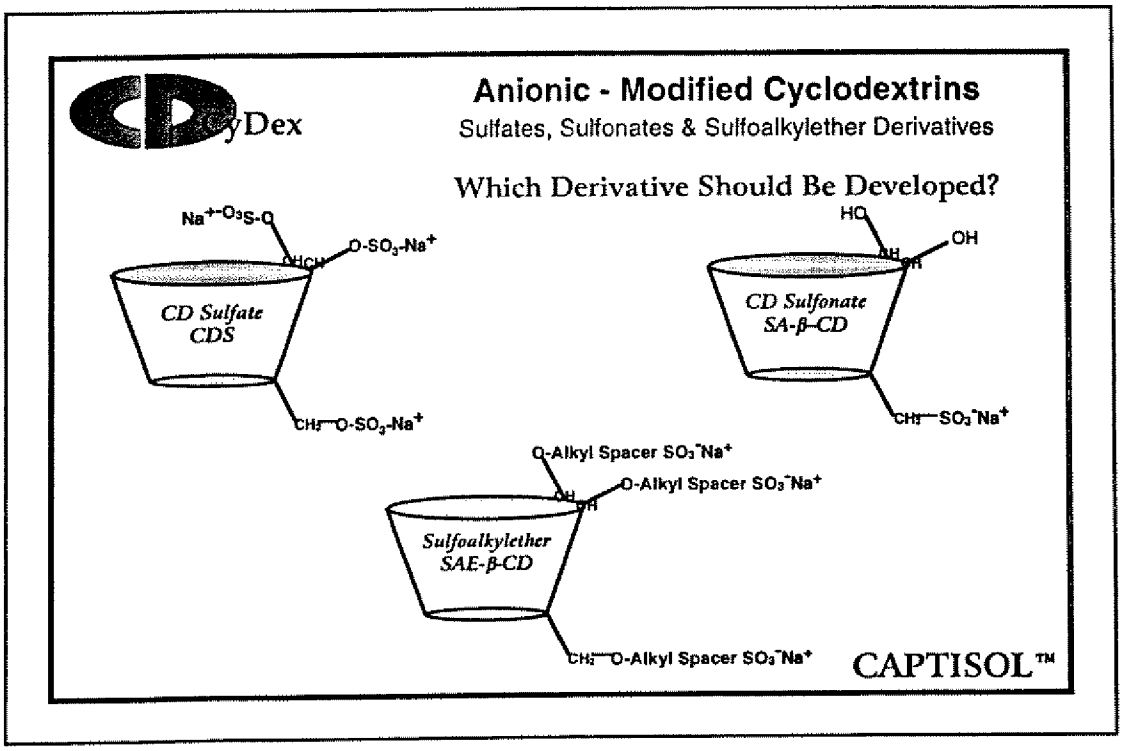
The parent cyclodextrins can not be used in parenteral formulations because upon systemic administration, β-CD causes extensive hemolysis, and dramatic nephrotoxicity. This toxicity is thought to be due to accumulation of the β-CD in the renal tubule cells where either the CD or the CD:cholesterol complex precipitates and is observed histologically as acicular crystals. How this disrupts cellular function in unknown but systemic administration of the parent cyclodextrins is not possible. Therefore, researchers have explored modifications of the parent cyclodextrins in search of a derivative that is safe to use systemically.



The interest in the use of CDs for parenteral formulations spurred a number of research efforts in the development of derivatized CDs that would have a good systemic safety profiles while retaining the functional complexation characteristics of the parent cyclodextrins.

Several laboratories explored the introduction of hydroxypropyl substituents by the reaction of β-CD with propylene oxide. This reaction introduces the HP substituent randomly at the 2, 3, and 6 positions and the manufacturing process can be controlled to produce materials with different degrees of substitution. The introduction of an increasing number of HP substituents does indeed increase the intrinsic water solubility of the derivative. The increased aqueous solubility of these HP-CD derivatives does provide for a CD material that exhibits a much better safety profile that the parent cyclodextrin.

HP-CD preparations with a DS of 4 or greater exhibit aqueous solubilities in excess of 50% (wt/vol). However, the introduction of a single HP substituent actually produces a material with a solubility less than the parent CD! Therefore, to be useful, the HP-CD material must have higher levels of substitution but, unfortunately as the DS increases, the complexation capability of the HP-CD has been observed to decrease. This is thought to result from steric crowding of the cavity opening by the HP substituents.



The design of Captisol is based on an evaluation of the structural criteria necessary to have maximum solubility and to capitalize on the efficient excretion of ionic metabolites by the kidney into the urine. Three different families of anionic cyclodextrin derivatives were evaluated in the search for a safe but functional CD derivative. The anionic substituents studied were salts of sulfur based acids. These anions should remain unprotonated throughout the pH range used in pharmaceutical formulations. Therefore, pH changes should not affect the properties of the CD although they may affect the complexation if the drug's state is affected by pH.

Sulfate Derivatives

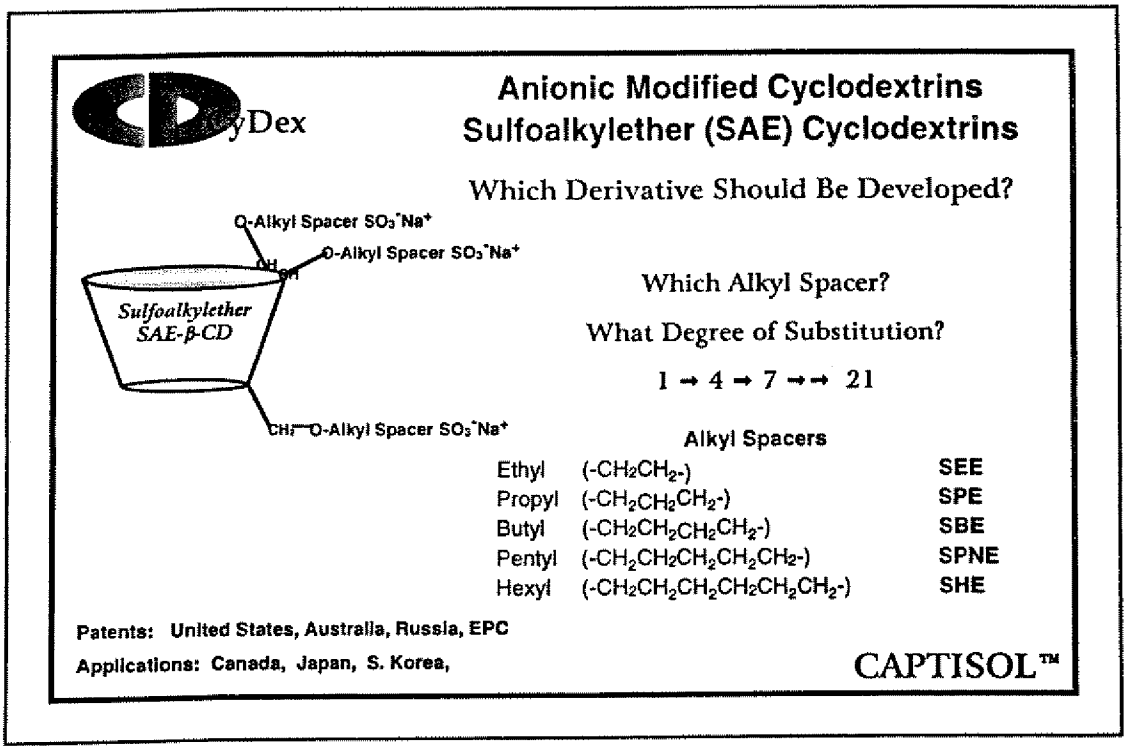
The first family was a directly sulfated CD. These molecules are easy to produce chemically and have the anionic sulfate group randomly distributed at the C2, 3, and 6 positions. The substituent is attached to the carbohydrate via an ester linkage which may be metabolically unstable *in-vivo*. One feature to note about this family is that the negative charge of the substituent is in close proximity to the carbohydrate backbone.

Sulfonate Derivatives

The directly sulfonated CDs were the second family of compounds studied. The substituent was introduced only at the C-6 position via several synthetic steps. The substituent was attached to the CD via a metabolically stable C-S bond. Like the sulfate derivatives, the sulfonates have the negative charge of the substituent in close proximity to the carbohydrate backbone.

Sulfoalkylether Derivatives

In the last family, the sulfonate anion is attached to a neutral alkyl spacer unit which links to the CD structure by a metabolically stable ether linkage. This group of compounds differ from the first two families in that the anionic charge is spaced away from the carbohydrate backbone by the alkyl groups.

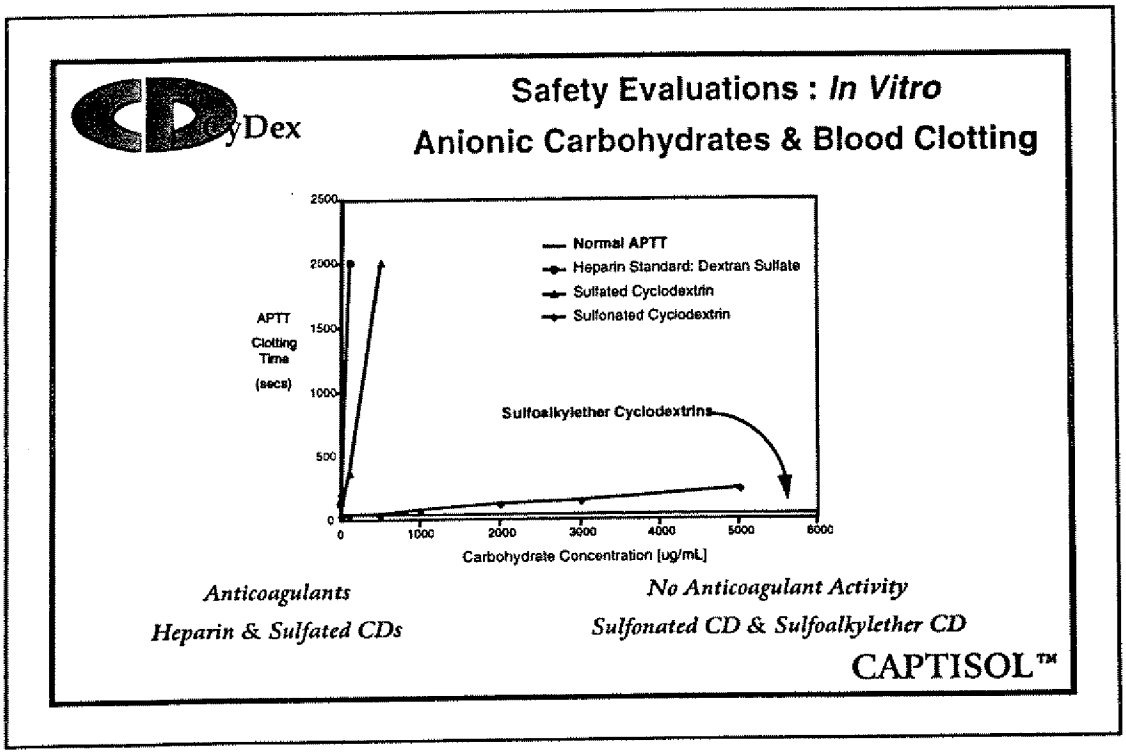


Sulfoalkylether Cyclodextrins

In selecting the appropriate anionic CD to develop, representatives from all three families (the sulfate, sulfonate and sulfoalkylether derivatives) were studied. In all of these families, the degree of substitution needed to be considered and optimized. In the sulfoalkylether family, the type of alkyl spacer unit was considered. The alkyl groups varied in chain length from a two carbon ethyl group to a six carbon hexyl group.

All of these sulfoalkylether cyclodextrins have been patented in the United States, Australia and Russia. The issued patents are composition of matter, as well as, use patents. The composition of matter claims cover the ethyl through hexyl groups, substitution levels from 1-21 and derivatives of all of the parent cyclodextrin.

The EPC patent has been accepted and is under public notice before being issued. Patent applications are waiting for review in Canada, South Korea and Japan.

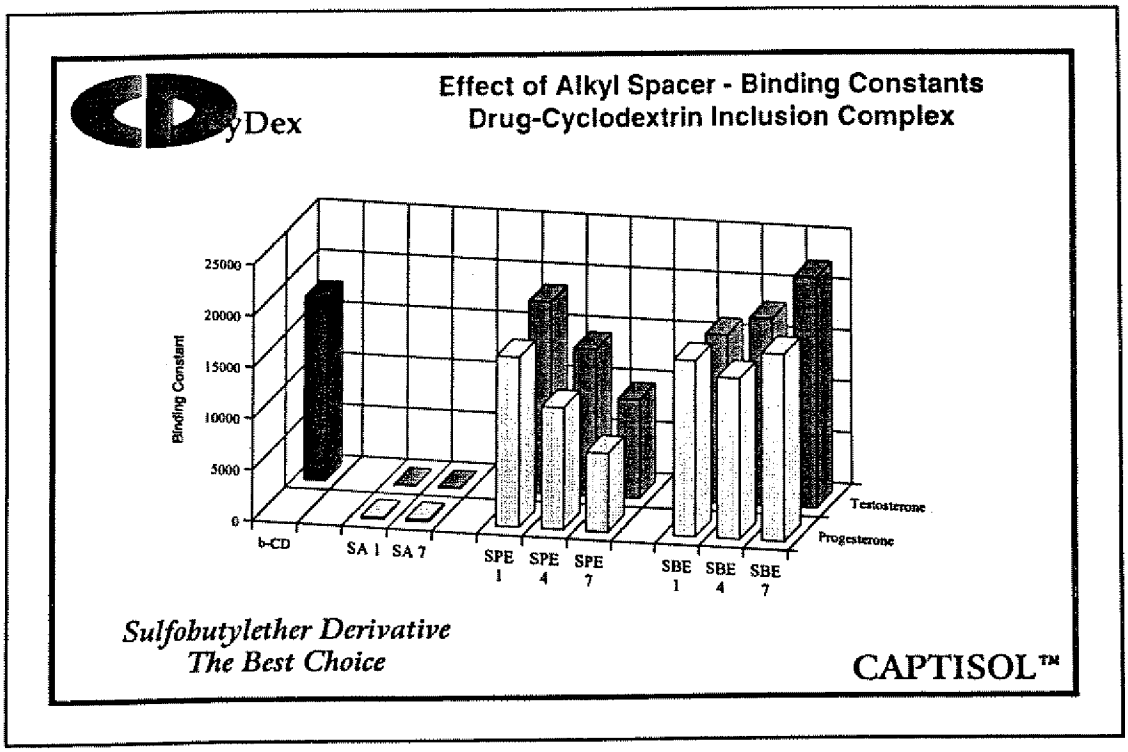


In evaluating the three anionic CD derivative families, safety considerations are always most important. The materials must be safe and devoid of any pharmacological activity. One pharmacological activity that has been observed with other anionic carbohydrates, is their effect on blood clotting times. Heparin, a sulfated linear carbohydrate is a well known anticoagulant.

The anionic CD derivatives were evaluated for their anticoagulant properties using a classical clinical assay, the Activated Partial Thromboplastin Time assay (APTT). The steep increase in clotting time with only µg/mL solution of a heparin standard (dextran sulfate) was also observed with the sulfated cyclodextrin.

However, the sulfonated cyclodextrins show a much reduced effect and the sulfoalkylether cyclodextrins exhibited no effect on the normal clotting time of blood even an concentrations up to 6000 µg/mL. [This concentration is reflective of the highest feasible dose administered and diluted in a human's plasma blood volume.]

These results suggested that only the sulfonate and sulfoalkylether derivatives would be suitable to pursue as the inactive formulation excipient.

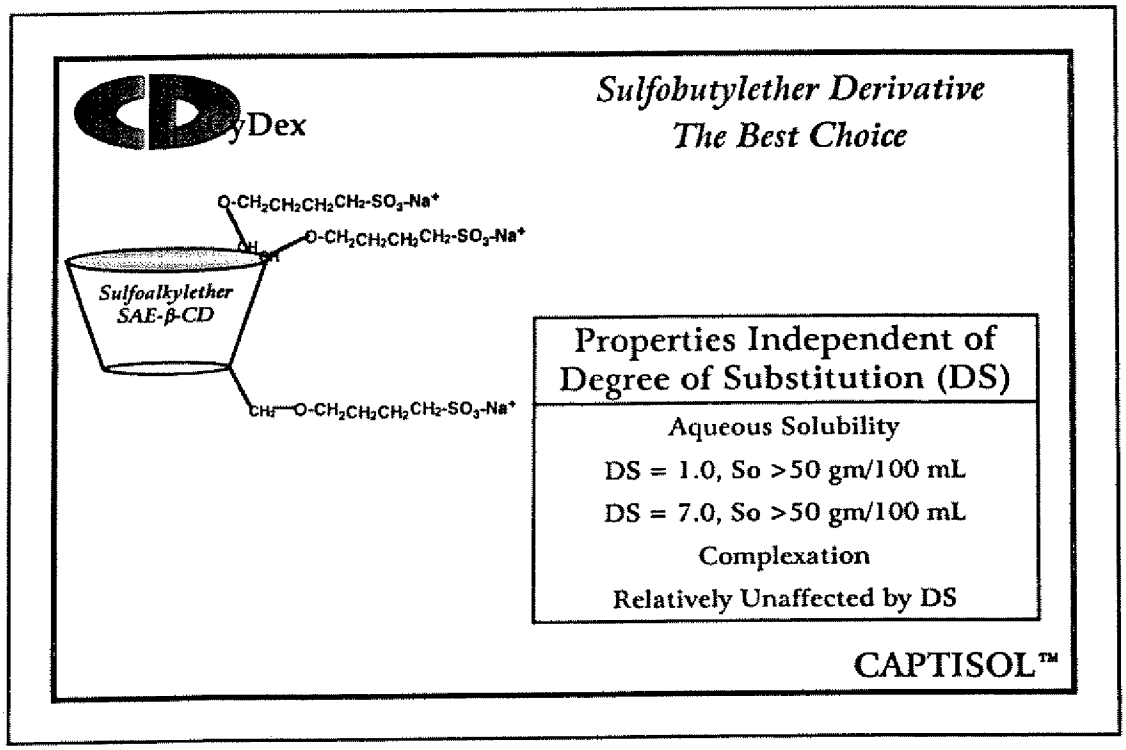


Ideally, the complexation capability of the cyclodextrin cavity should not be compromised by the derivatization process. However, typically the introduction of a substituent on the parent cyclodextrin results in a diminishing of the complexation capacity. In addition, for many of the neutral substituents, increasing the degree of substitution often decreases complexation even further possibly due to steric crowding of the cavity opening. This was noted for the HP-CD.

To evaluate the complexation of the anionic derivatives, the binding constants for the complexation of two hydrophobic and water insoluble steroids (testosterone and progesterone) were studied. The directly sulfated or sulfonated derivatives (both noted as SA-β-CD) showed complete loss of binding capability whether the degree of substitution was 1 or 7. Apparently, the proximity of the anionic charge close to the carbohydrate backbone and cavity environment eliminates the favorable thermodynamics driving the complexation.

Spacing the negative charge away from the CD backbone through the use of the 3-carbon propyl space unit verifies the impact of the proximity of charge on complexation. When the derivative bears only one substituent (SPE1-β-CD), the binding constants for the drugs rival that observed with the parent beta-CD. However, as the degree of substitution increases to 4 (SPE4-β-CD) and seven (SPE7-β-CD), the binding constants decrease. The increase in charge density from a mono- to tetra- to hepta- anion can not be lessened completely by the propyl spacer.

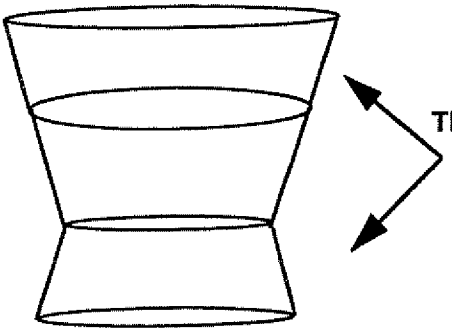
The sulfobutylether derivatives, however, do not display a change in complexation capability with a change in the degree of substitution. Therefore, the SBE-CD family was pursued further. The question remaining to be answered is, "What level of substitution should be introduced for optimal safety and functional performance."



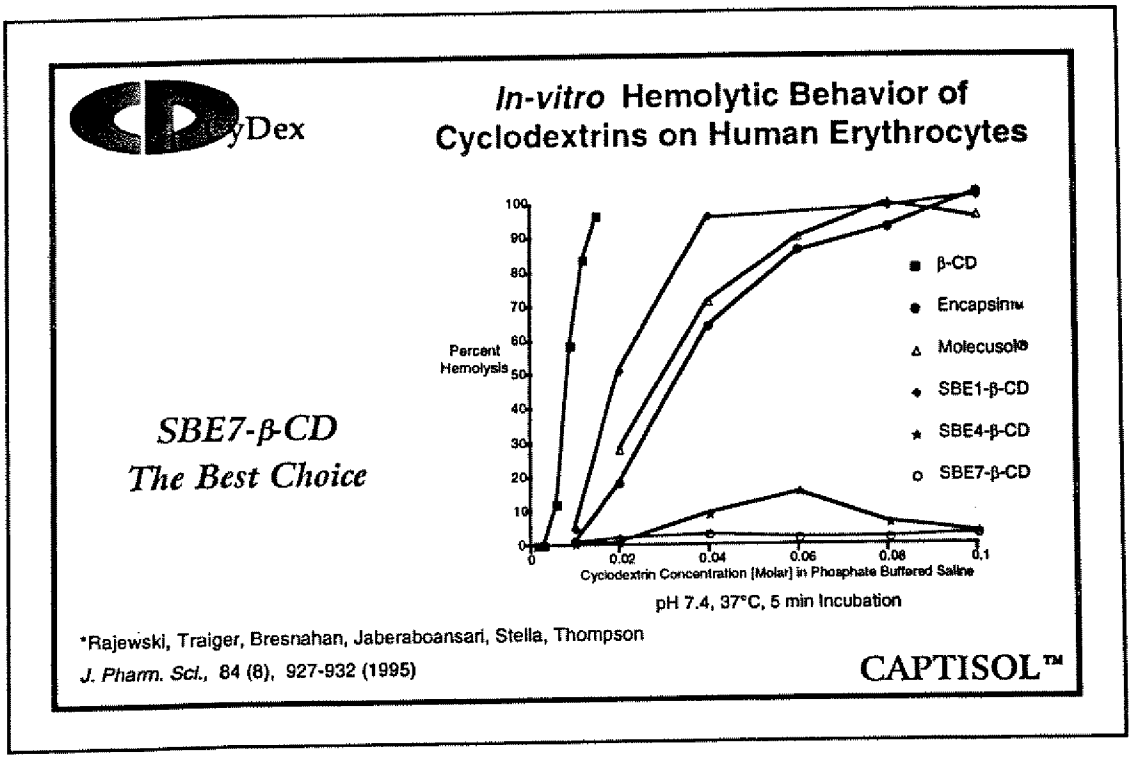
The sulfobutylether derivatives exhibit functional properties that are relatively independent of the degree of substitution. The derivatives exhibit favorable water solubilities at low and high degrees of substitution.

The complexation capability is relatively unaffected by the degree of substitution. For these charged derivatives, increasing the DS does not appear to exert a negative impact on complexation.

The steric hindrance, observed with increasing the DS for HP-CD preparations, is not observed with the SBE-substituents. The SBE-groups may possibly orient themselves up and away from the cavity. The hydrophobic butyl chains may align to minimize interactions with the aqueous solution similar to the process observed in micelle formation. If the butyl groups align, the sulfonate anions would be brought together but due to electrostatic repulsions the anionic sulfonates should spread out. This would provide for elongated hydrophobic cavity with a clear opening to the cavity.



SBE substituents with butyl groups aligned. The sulfonate anions at the end of each butyl chain repel each other causing the substituents to spread out. This elongates the cavity and maintains the opening.



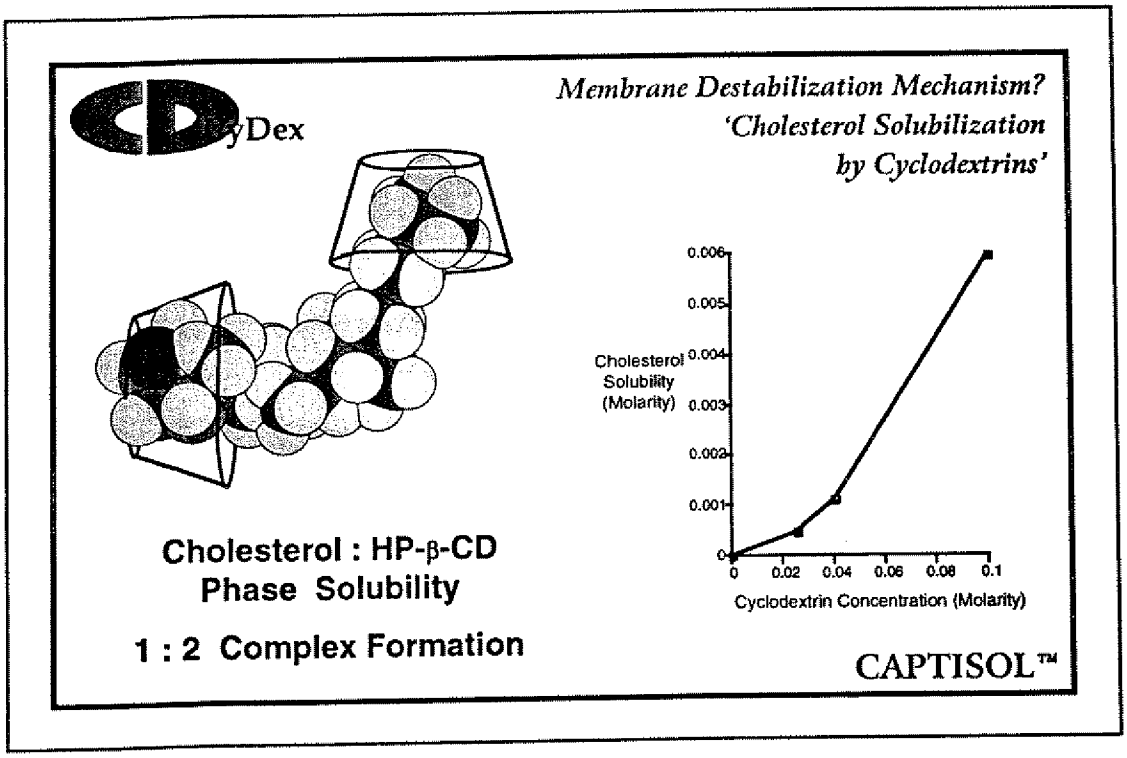
In determining the optimal degree of substitution for the sulfobutylether derivative, we once again return to the safety considerations. In order to be used parenterally, the SBE-CD must be devoid of any traces of the β-CD used as a raw material for the derivatization.

The renal toxicity of the parent β-CD is thought to be due to precipitation of the CD alone or in combination with hydrophobic membrane components such as cholesterol. The parent cyclodextrins are observed to interact with cellular membranes, extracting cholesterol. The removal of cholesterol from the membrane weakens the membrane structure and this ultimately results in the lysis of the cell. An *in-vitro* assay using erythrocytes is used to compare the membrane damaging characteristics of various CDs.

In the hemolysis curves above, the ability of β-CD to lysis cells is observed at low molar [M] concentrations. In the center of the diagram are the hemolysis curves for two brands of hydroxypropyl cyclodextrin that vary in their degree of substitution. Encapsin has a DS ≈ 4 while Molecusol has a DS ≈ 8. Although the DS varies in these two materials , their hemolytic behavior is almost equivalent.

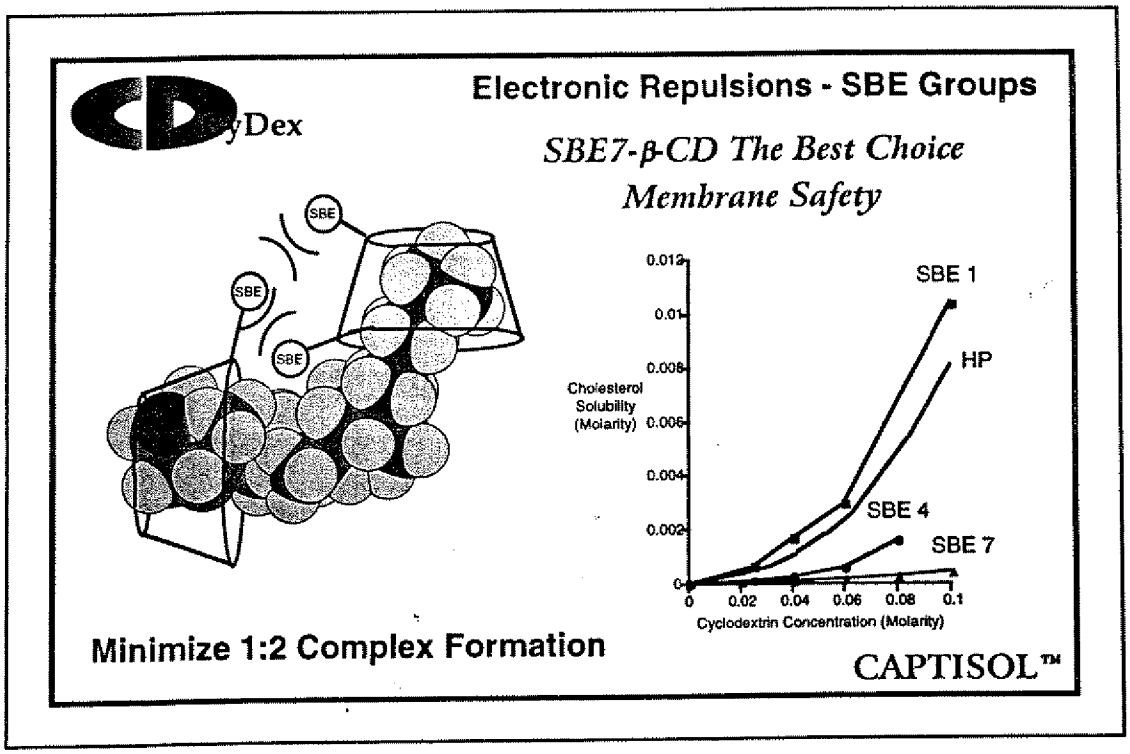
Also in the center of the graph is the hemolysis curve for the mono- substituted sulfobutylether derivative of β-CD, SBE1-β-CD. This preparation exhibits membrane damaging behavior similar to that observed for the two different HP-CD preparations and SBE1-β-CD may actually be slightly more damaging.

However, for the sulfobutylether derivatives, as the degree of substitution is increased, the membrane damaging effects are decreased. In fact, under the conditions studied, the SBE7-β-CD exhibited no lysis of the human red blood cells.



The membrane damaging effects of the cyclodextrins is thought to result from the extraction of cholesterol and other hydrophobic components from the membrane. These cause a destabilization that ultimately weakens the membrane structure and ultimately results in cellular lysis.

For the solubilization of cholesterol by β-CD or HP-β-CD, two cyclodextrin molecules 'string' onto the molecule. This 1:2 complexation is reflected by the upward curvature (Ap) behavior seen in the phase solubility diagram.



The sulfobutylether derivatives do not effectively participate in 1:2 complexations. This may be due to electronic repulsions between first SBE-CD molecule to encapsulate the cholesterol and the second incoming negatively charged SBE-CD.

This repulsion is minimized at low degrees of substitution. In fact, the mono-substituted SBE-CD more effectively forms 1:2 complexes with cholesterol than observed with the HP-CDs. This may explain the similarities in membrane damaging effects observed in the hemolysis studies.

However, as the number of SBE-groups increases, the electronic repulsions that limit 1:2 complexation increase. The SBE7-β-CD is able to solubilize a small amount of cholesterol from 1:1 complexation but is very ineffective compared to the lower substituted derivatives. This mirrors again the hemolytic order for the different types of CDs.

The method to eliminate residual β-CD without any costly purification steps is to push the derivatization to higher levels of substitution. For a preparation of SBE7-β-CD, no β-CD or SBE1-β-CD are present in the preparation.

Therefore, SBE7-β-CD derivative appeared to offer the best features of safety, and functionality. SBE7-β-CD was chosen as the material to develop as the drug carrier system, Captisol.

CyDex

SBE7-β-CD : CAPTISOL™

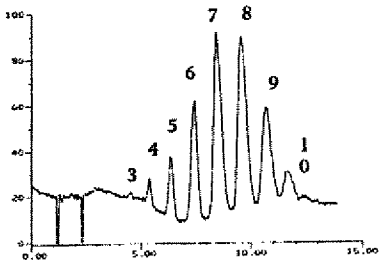
A New Drug Formulation System

Manufacture: 100 kg GMP Clinical Material
Stability: Solid State & Solution > 12 months
Specifications:

Identity
- IR, NMR, Specific Rotation
- Substitution Envelope (CE)

Impurities
- Residual β-CD, Sultone
- Sultone Side Products
- Chloride
- Heavy Metals

Microbiology
- 'Pyrogen-free'



Capillary Electropherogram* (CE) of Captisol™

*Tait, Skanchy, Thompson, Chetwyn, Dunshee, Rajewski, Stella, Stobaugh
J. Pharm. Biomed. Anal., 10 (9), 615-622 (1992)

CAPTISOL™

Captisol (SBE7-β-CD) is a well characterized, reproducible composite mixture, not a single molecular entity. Since β-cyclodextrin is a ring structure of seven glucopyranose units, with three derivatizable hydroxyl groups per sugar, the sulfobutylether derivatization can occur at 21 different sites on the molecule,

The manufacturing process generates a composite mixture containing several fractions with different degrees of SBE substitution, and a number of different SBE-β-CD isomers (regio- and positional) within a given fraction.

Analysis of Captisol by capillary electrophoresis shows a profile similar to that shown above. CyDex's scaled-up production method has yielded the same purity profile and complexational potency for multiple lots. Specifications have been established for the characterization of Captisol in terms of Identity, Assay and all potential Impurity profiles. Captisol is provided as a 'pyrogen-free' solid suitable for use in intravenous clinical studies.

Captisol has exhibited solid and solution state stability (research studies*) for well over 12 months. Captisol is currently on stability studies which meet ICH Guidelines.

*SBE7-β-CD Bulk Solid (5°C, 30°C, 40°C, 40°C/75% Relative Humidity and 2000 lux/25°C) SBE-β-CD absorbs water at 75% humidity but otherwise is stable beyond 12 months

*SBE7-β-CD Aqueous Solution (300 mg/ml aqueous solutions of SBE-β-CD, stored in 50 ml clear glass vials at 25°C, 30°C, 40°C and 50°C) SBE7-β-CD is stable beyond 12 months

CDyDex

Summary of Preclinical Data

Outline of Preclinical Animal Safety Studies

General Pharmacology
Pharmacokinetics and Disposition
Genotoxicity Studies
I. V. Rodent Studies - 14day, 30day, 180 day
I. V. Dog Studies - 14day, 30day, 180 day
I. V. Reproductive Safety: Segments I, II, III: Rats & Rabbits

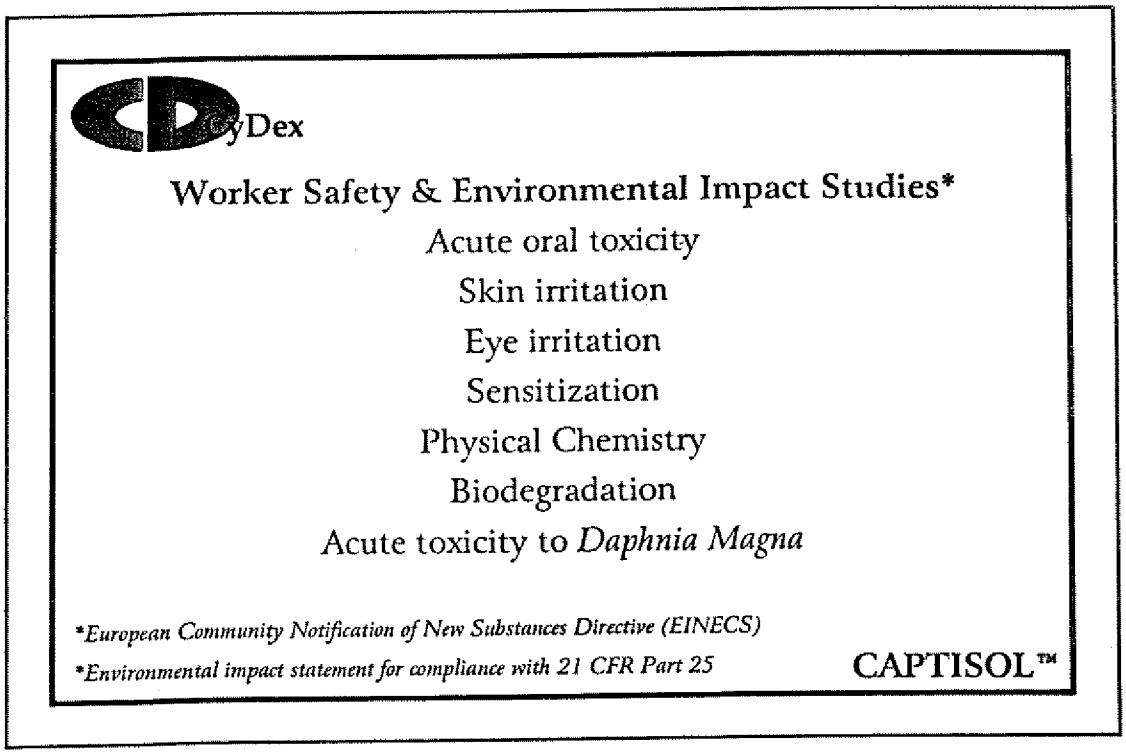
CAPTISOL™

The preclinical animal studies completed to date are summarized above. An entire battery of pharmacology and genotoxicity studies have been conducted and show no evidence of pharmacological activity or mutagenic behavior.

Intravenous administration of Captisol in mice, rats and dogs has been evaluated in single acute dosing through 6-month daily dosing studies. No toxic events were observed during the course of these studies.

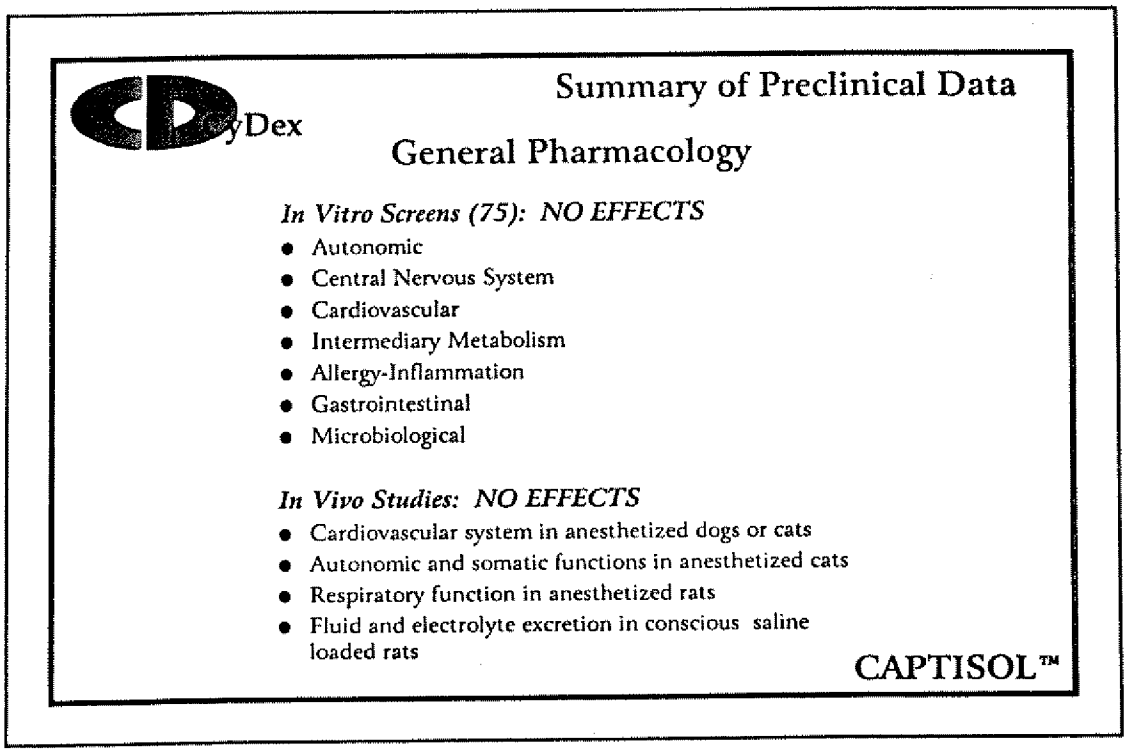
The pharmacokinetics of radiolabelled* Captisol show that the material is rapidly excreted unchanged in the urine. Captisol distributes only into the extracellular fluid and does not accumulate in any tissue reservoir even upon repeated dosing. Captisol is excreted at the glomerular filtration rate in rats, dogs and humans.

*(^{14}C label in the SBE group on the carbon attached to the cyclodextrin structure)



In order to transport bulk Captisol in Europe and to satisfy the environmental impact section of NDA filings in the USA, a safety data package has been assembled to insure worker safety and environmental concerns.

The current data package satisfies the EINECS requirements for transporting bulk Captisol in the EC.



An essential feature of any drug carrier system is that the carrier molecule be pharmacologically inactive. Captisol meets this characteristic. SBE-CDs have been evaluated in 75 *in-vitro* pharmacology screens and these anionic CDs exhibit no pharmacological activity.

In vivo studies have provided further evidence of the inactivity of Captisol on the cardiovascular system, autonomic and somatic functions, respiratory function and fluid and electrolyte balance.

Genotoxicity Studies - All Tests Negative

Assay	Test System	Highest dose/concentration
Microbial reverse mutation	*Salmonella typhimurium* strains TA98, TA100 TA1535, TA1537	5 mg/plate
	Escherichia coli strain WP2 uvrApKM101	5 mg/plate
Mammalian cell gene mutation	CHO/HGPRT	5000 μg/ml
In vitro cytogenetics	Human lymphocytes	5000 μg/ml
In vivo cytogenetics (mouse micronucleus)	Male mouse bone marrow	3000 mg/kg/day for 3 days

In vitro tests were performed with and without S9 metabolic activation.

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The extensive battery of assays have been conducted to determine the genotoxic or mutagenic potential of Captisol. These studies satisfy registration requirements set by all global authorities.

In-vitro and *in-vivo* assays recorded no effects for Captisol when studied over a series of concentrations with the highest concentrations shown in the table above. These results suggest that Captisol is not expected to elicit mutagenic or genotoxic effects *in-vivo.*

Summary of Preclinical I. V. Safety Data

CyDex

Study Type	Species	Dose Level (mg/kg body weight/day)
Single dose	Mouse/Swiss CD1	2000
Single dose	Rat/Sprague-Dawley	2000
14 days RF	Rat/Sprague-Dawley	160-240-600-1500
14 days RF	Dog/Beagle	160-240-750
1month	Rat/Sprague-Dawley	160*-240-320*
		40 - 80 - 160
Maximum Tolerated Dose Study		300**-1000**- 3000**
1month	Dog/Beagle	100*-200-300*
		30 - 60 - 120
Maximum Tolerated Dose Study		300**-750**- 1500**
6 month	Rat/Sprague-Dawley	200 - 320 - 600
6 month	Dog/Beagle	150 - 300 - 600

*Reversibility: * 1, **2 & **5 months*

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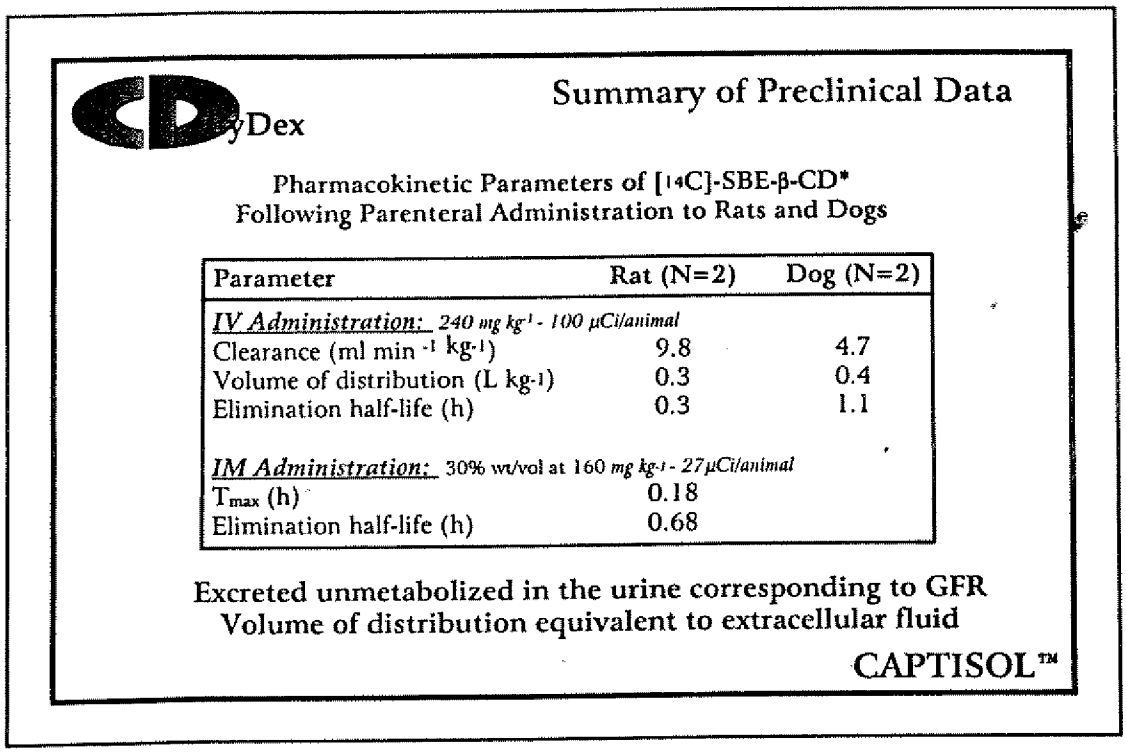
The table above summarizes the preclinical intravenous safety studies that are complete as of April 1, 1996. No adverse or toxic events were observed in any of the studies.

The number of animals per study (per gender group) meet the standard practice of the industry to satisfy global registrations*.

* "Harmonization of Guidelines for Toxicity Testing of Pharmaceuticals by 1992", Spied, Lumley & Walker, *Regulatory Toxicology and Pharmacology*, 12, 179-211 (1990)

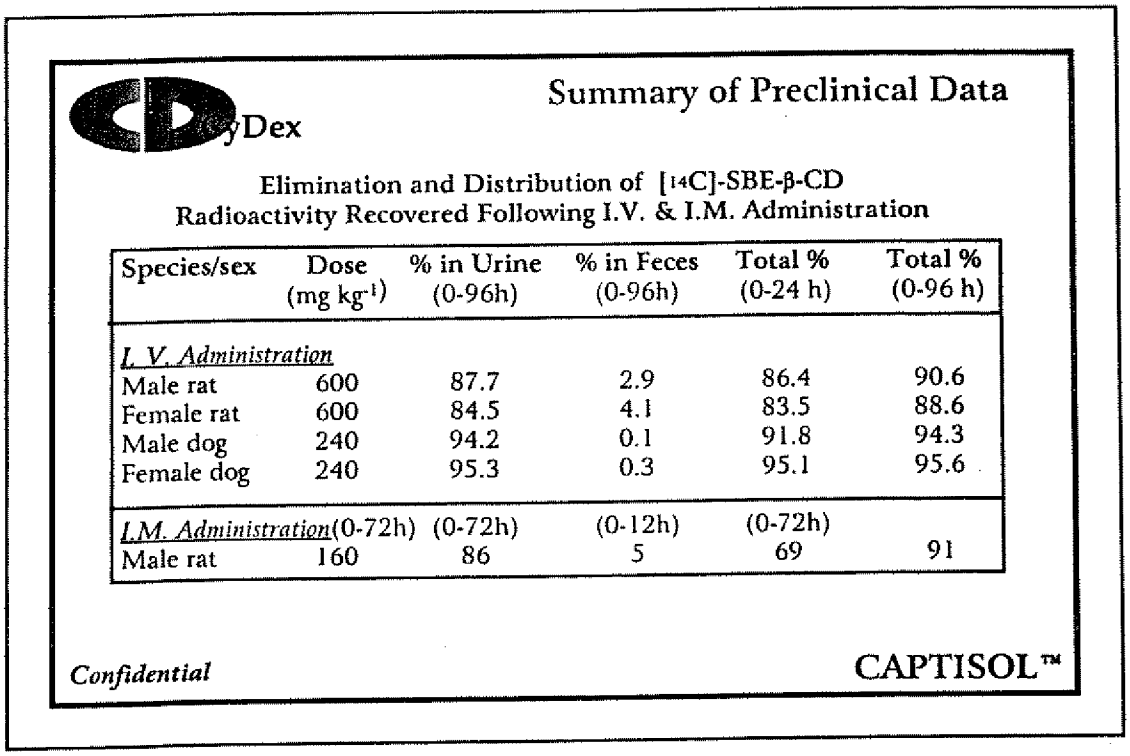


[14C]-SBE7-β-CD has been prepared in a manner consistent with the production of Captisol. The [14C] label is incorporated in the SBE group, specifically at the carbon attached to the CD structure. Studies have confirmed that SBE7-β-CD is not metabolized during *in-vivo* administration. There is no metabolism of the SBE7-β-CD that results in loss of the radiolabelled group from the CD.

The i.v. administration of [14C]-SBE7-β-CD to rats and dogs results in clearance rates and elimination half lives suggesting that the CD is rapidly eliminated by the kidneys at a rate approximating the glomerular filtration in each species.

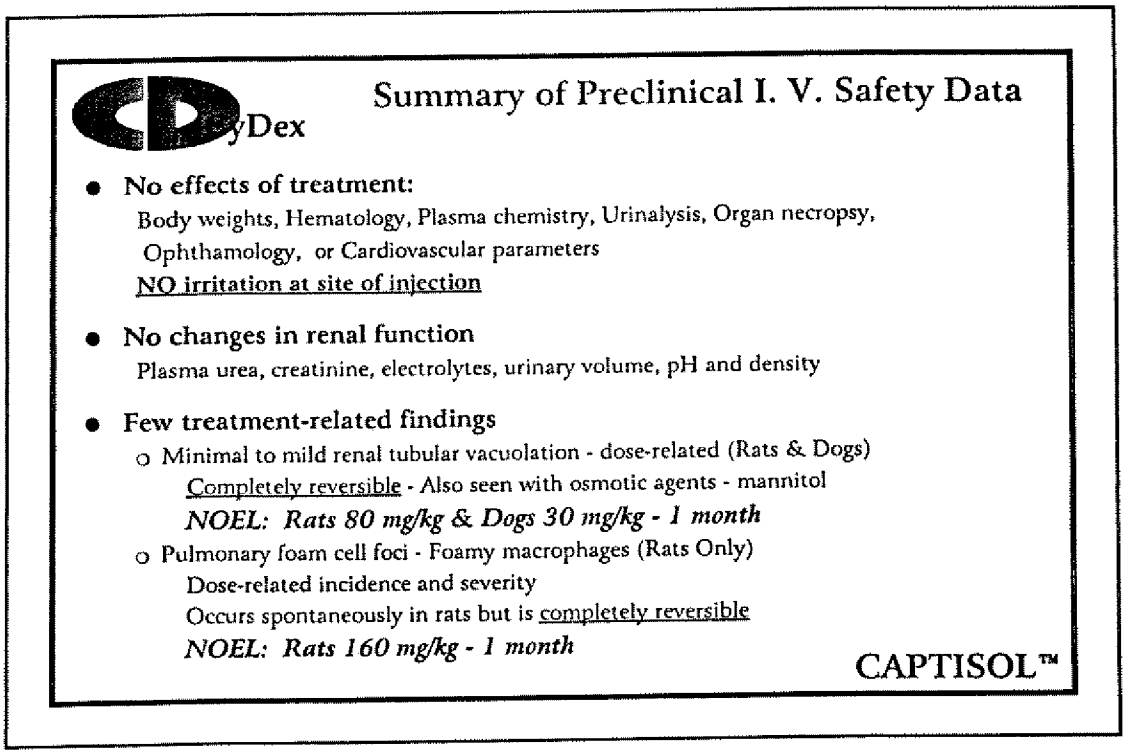
The volumes of distribution indicate the SBE7-β-CD is confined to the circulatory system and the extracellular fluid. Autoradiography studies have confirmed that SBE7-β-CD does not concentrate in any tissue but the time course follows that expected for a material cleared by the kidneys.

For the i.m. administation of [14C]-SBE7-β-CD to rats, the T max of 0.18 hr (11 min) shows that Captisol is rapidly absorbed from the injection site. The elimination half-life of 0.68 hr (41 min) shows that the material is rapidly cleared from the systemic circulation as expected from the i.v. results.



≈89% and 95% of the i.v. doses of [14C]-SBE7-β-CD were recovered from rats and dogs. The majority of the excretion occurred by the urinary elimination within 24 hours. A small percentage of the dose (≈3% in rats and only 0.2% in dogs) was observed in the feces indicating that biliary excretion is minimal.

The excretion paths (both urinary and fecal) for i.m. administration of Captisol were comparable to those observed in the i.v. dosing studies.



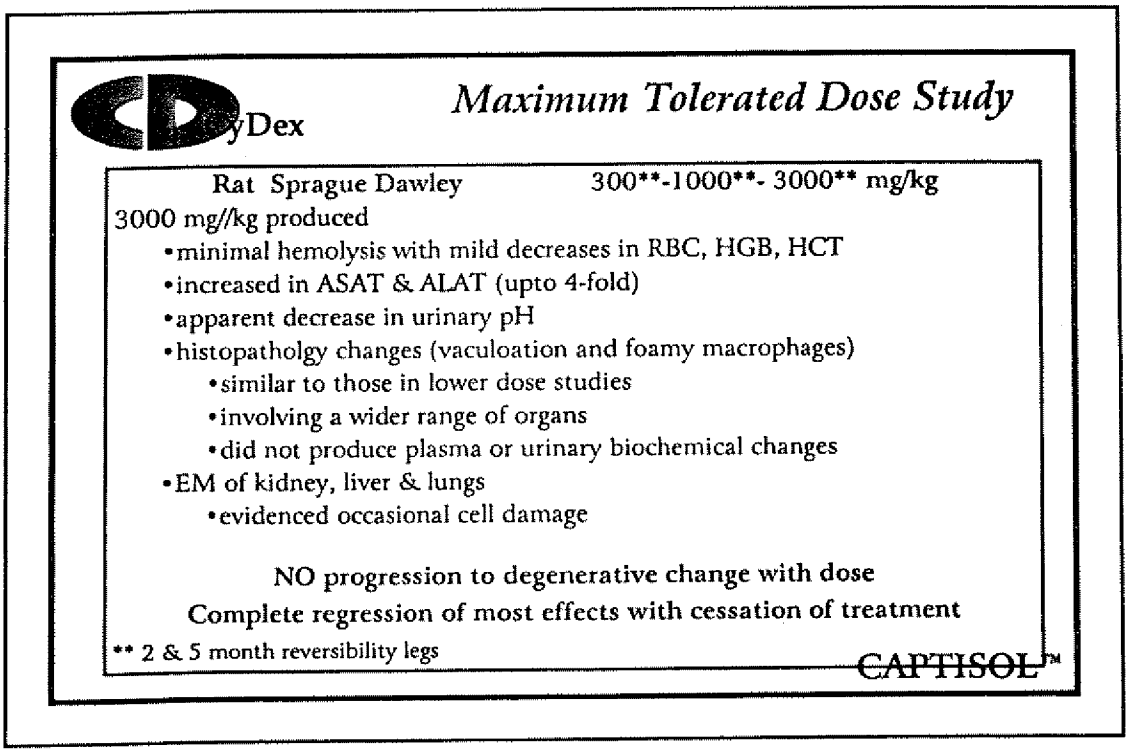
All of the animal safety studies have shown Captisol to be safe for intravenous administration. Captisol was shown not to affect the general health of the animals. There were no effects of the treatment on body weight gains, blood chemistry, urinalysis or gross tissue necropsy. Captisol administration causes no changes in renal function as measured by the extensive battery of tests shown above.

This is in contrast to the effect of HP-β-CD studied under similar conditions. The product literature on HP-β-CD has reported that this derivative causes body weights to decrease, plasma chemistries to change and the spleen to become hyperplasic. By these observations, Captisol appears to be better tolerated than HP-β-CD for systemic administration.

Although necropsy of the organs (Captisol treatment) showed no gross abnormalities, histological evaluation of the tissues demonstrated several changes as noted above. The vacuolation observed in the renal tubular cells is characteristic of an adaptive response to the presence of an osmotic agent. A similar response has been observed upon administration of high concentrations of a widely used excipient, mannitol. These vacuoles do not contain the acicular crystals that are characteristic of the precipitates observed during the irreversible damage caused by β-CD. Renal Vacuolation had a NOEL (No Effect Level) of 80 mg/kg in rats and 30 mg/kg in dogs. Vacuolation of the kidneys is almost completely reversible upon cessation of treatment.

The vacuolation of the urinary bladder epithelia in the dog study was not dose related and also occurred in some control animals. This effect is not thought to be related to Captisol. The increase in pulmonary foam cell foci in the rat study was dose related but was completely reversible upon cessation of treatment. Foam cell foci had a NOEL of 160 mg/kg in rats.

None of these changes are considered to be of toxicological significance. NOEL for Captisol in dogs at 30 mg/kg which would equate to a 4.2 gm dose in a 70 kg human patient.



With the systemic safety of Captisol established, additional safety studies have been initiated or are under consideration. The reproductive safety studies are currently in progress in the rabbit and rat.

A 30-day studies to determine a maximal tolerated dose is being considered as a result of a meeting between CyDex and the FDA in January 1995. The FDA representatives agreed that the results observed during the preclinical studies were not toxic events. They suggested however that higher doses should be administered in search of a toxic event. These studies are being designed.

Due to an interest in the use of Captisol formulations for other routes of administration, CyDex is considering safety studies by different routes (oral, nasal, ophthalmic, pulmonary etc).

Maximum Tolerated Dose Study

CyDex

Dog: Beagle 300**-750**- 1500** mg/kg

- Vacuolation in renal and bladder epithelium, hepatocytes
- Foamy Macrophages in liver & lymph nodes

Only new finding vs lower dose studies
was presence of foamy macrophages in lymph nodes

NO progression to degenerative change with dose

Regression of effects with cessation of treatment
was extensive but not complete for the 750 and 1500 mg/kg

** 2 & 5 month reversibility legs

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I. V. Reproductive Safety

Study Type	Species	Dose Level (mg/kg body weight/day)
Fertility	**Rat**	**100 - 400 - 1500**
Decrease in maternal body weight & food consumption at 1.5 g/kg No effects on fertility (both sexes) No effects on early embryonic development		
Teratology	**Rat**	**100 - 600 - 3000**
Peri & Postnatal Development	**Rat**	**100 - 700 - 3000**
Decrease in maternal body weight gain & food Consumption at 3000 mg/kg No effects on reproduction parameters No teratogenic effects		
Peri & Postnatal Development	**Rabbit**	**100 - 400 - 1500**
No maternal toxicity No effects on reproduction parameters No teratogenic effect		

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I. V. Preclinical Safety Conclusions

- Captisol is not genotoxic
- Noteworthy effect of treatment
  - Renal tubular vacuolation
  - Foamy macrophages in liver & lungs
- Only borderline toxicity in Rats at 3 gm/kg/day
- No toxicity with Dogs at 1.5 gm/kg/day
- No effect on male or female fertility
- No teratogenic effects

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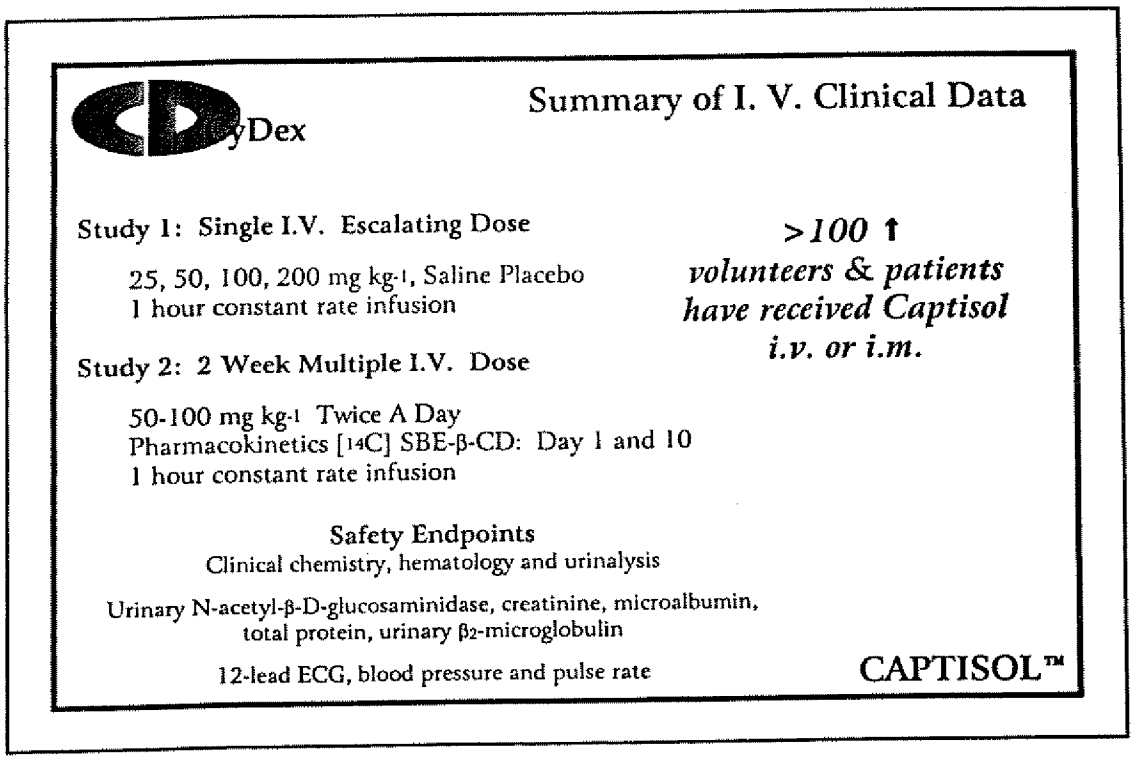
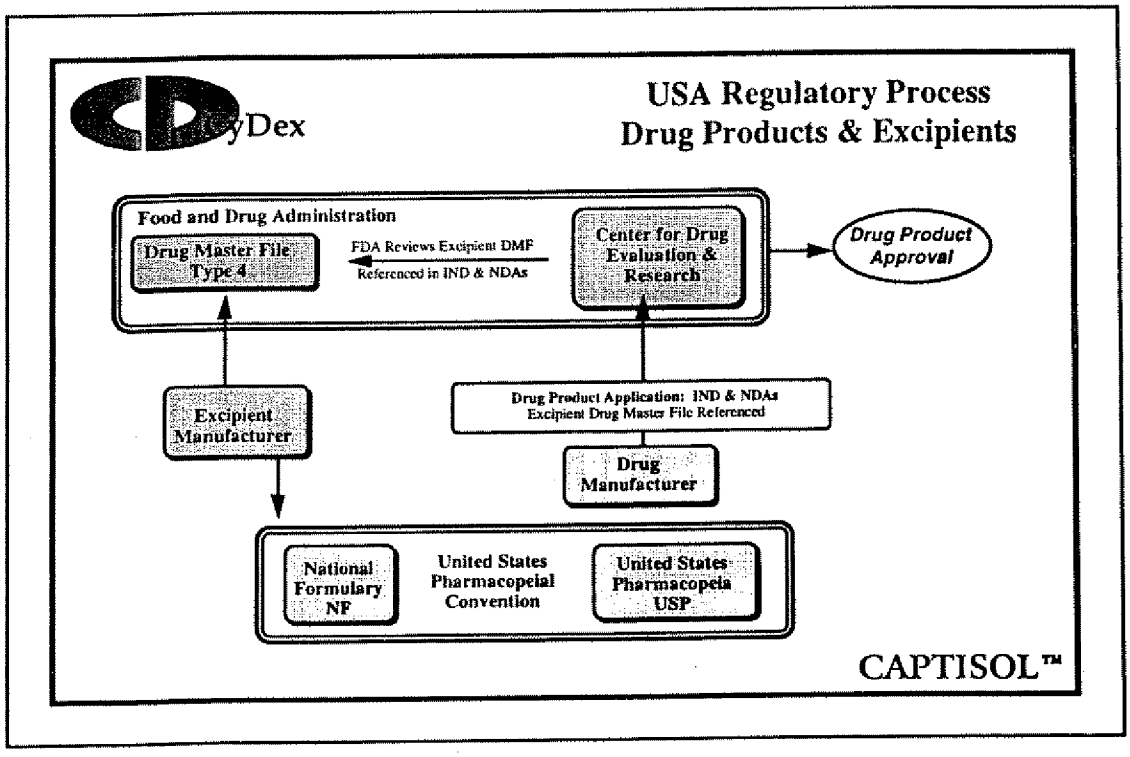


The first human clinical trials on Captisol alone are summarized above. In the first study, single doses of Captisol were administered to healthy males in a 1-hour infusion. The doses were administered in an escalating manner to each volunteer. No effects were observed for the treatment.

In the second study, the human volunteers were dosed twice a day. The pharmacokinetics of Captisol were measured on day 1 and day 10 to determine if multiple doses would affect the pharmacokinetics. No adverse effects were observed during the course of treatment and the pharmacokinetics were the same at the beginning and end of treatment.

Humans show rapid elimination of unmetabolized Captisol in the urine. The clearance and volume of distribution are consistent with elimination at the glomerular filtration rate and distribution only in the extracellular fluid compartment.



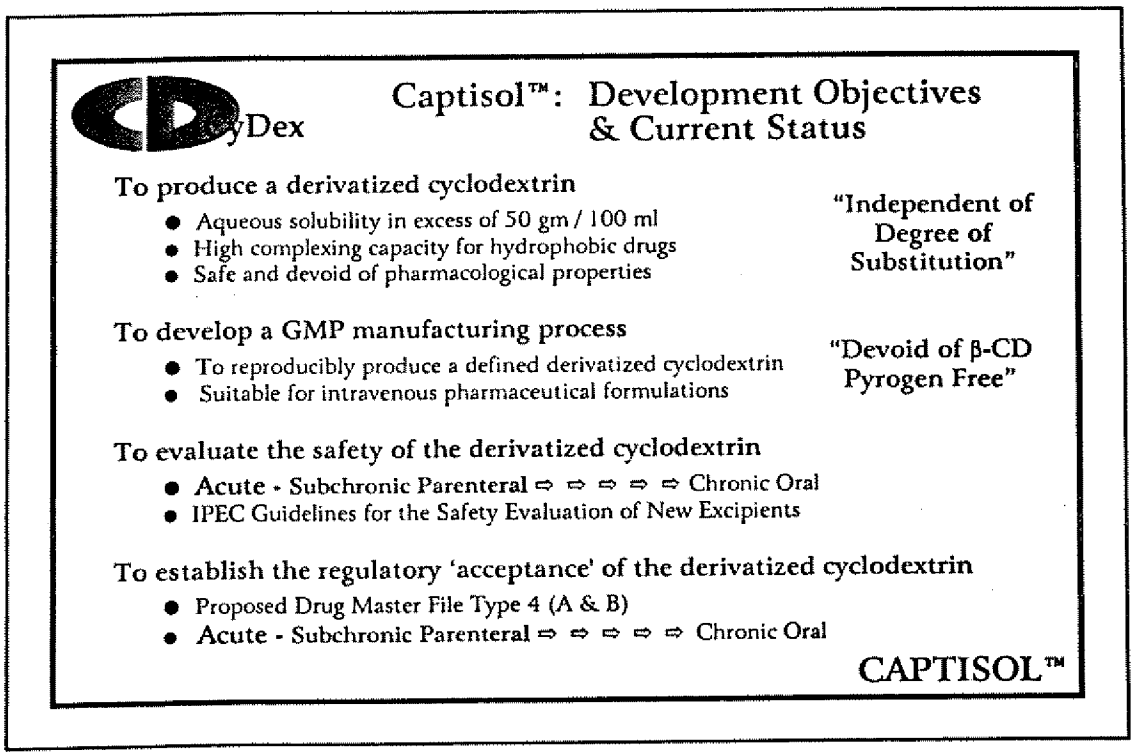
Captisol is considered a new excipient. The FDA and other global regulatory agencies review a new excipient only in relationship to the review of a drug formulation. Only the final drug product is approved by the FDA.

There is a perception that a process exists for the review and approval of a new excipient with the granting of 'GRAS' (Generally Recognized As Safe) status. However, the GRAS status applies only to food additives. The pharmaceutical industry often uses GRAS food additives in oral drug products assuming that the additive will cause no undue concerns for the formulation. This assumption is true if the excipient level does not exceed that approved for use in foods. Unfortunately, no process exists for review of excipients to be used by non-oral routes of administration, yet non-oral products account for 30% of the pharmaceutical sales in 1993.

Captisol is a new excipient and the FDA may request safety and clinical data for the drug:Captisol formulation and for Captisol alone. The preclinical and clinical safety data for Captisol alone is documented in a Drug Master File (DMF) that may be referenced by a licensee. CyDex's initial regulatory strategy focuses on establishing the use of Captisol as a drug carrier system for injectable formulations to be followed by further development of additional routes of administration.

The safety package for the use of Captisol in i.v. formulations has been developed as part of a licensing agreement with Pfizer Inc. Additional safety studies for other routes of administration will be added to the DMF as the data become available. CyDex retains ownership of these studies and the Drug Master File on Captisol and is able to share the information with prospective licensees after the execution of a confidential disclosure agreement.

CyDex met with the FDA (1/95) and presented the Chemistry, Manufacturing and Control section and Safety section of the DMF in support of the initial IND filings. The FDA comments at that meeting were supportive of the use of Captisol in clinical trials. The first IND for injectable formulation (i.v.) containing Captisol was submitted in Oct. 1995 and a second IND filing for a different drug product (i.m.) was submitted in the spring of 1996. Both clinical studies were authorized.

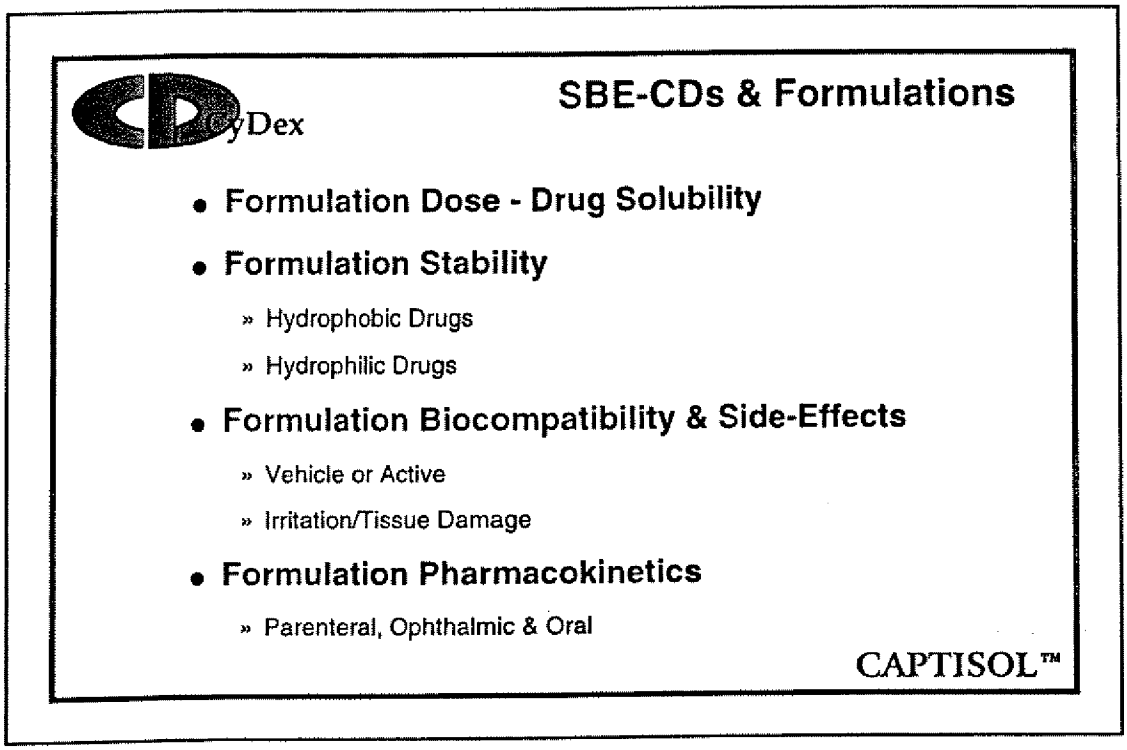


The purpose of this presentation is to explain the design of Captisol, the current status of the manufacturing process, and safety data package and the regulatory situation facing Captisol.

The design of Captisol focused on the three objectives listed at the top of the slide with the additional feature that these goals were to be attained independent of the degree of substitution of the derivatized CD. The necessity of this feature will be explained in subsequent slides.

CyDex's initial regulatory strategy focuses on establishing the use of Captisol as a drug carrier system for injectable formulations to be followed by additional routes of administration. Therefore a manufacturing process capable of producing a pyrogen-free material has been developed.

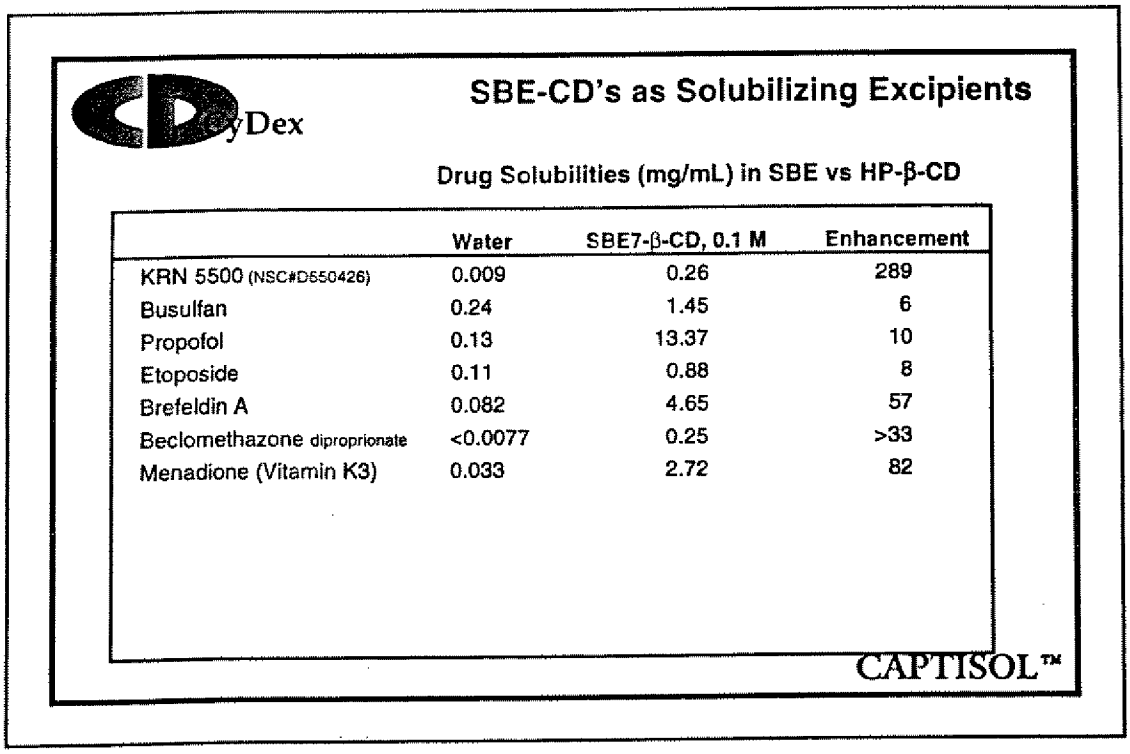
The safety package for the use of Captisol in parenteral formulations has been developed as part of a licensing agreement with Pfizer Inc. Additional safety studies for other routes of administration will be added to the DMF as the data become available. CyDex retains ownership of these studies and the Drug Master File on Captisol and is able to share the information with prospective licensees after the execution of a second confidential disclosure agreement.



This presentation explores the use of sulfobutylether cyclodextrin derivatives in drug formulations. SBE-CDs can be used to improve drug solubility and stability. Stability examples will be given for hydrophobic and hydrophilic drugs.

Examples will be given to show the biocompatibility of the SBE-CD vehicle and how complexation can reduce side-effects of the active such as tissue irritation.

Finally, the effective delivery of active drugs will be presented in terms of the pharmacokinetics of SBE-CD formulations for parenteral (i.v. and i.m.), ophthalmic and oral administration.



The table above shows several hydrophobic drugs exhibiting poor intrinsic water solubility. The solubility of the drugs in 0.1M solutions of HP-β-CD and SBE4-β-CD are shown in the second and third columns. (Although the data is shown for the tetra-substituted SBE-CD, the previous discussion has shown that the DS does not affect the complexation performance and Captisol has been shown to produce similar results to those seen in this table.)

Testosterone solubility increases from 0.01 to 20.20 mg/mL in a 0.1M SBE4-β-CD solution (≈15% wt/vol). This is a 2000 fold increase!

In the majority of cases, the SBE derivative is more effective as a solubilizing agent that the HP derivative by a factor of 1.5 to 2.

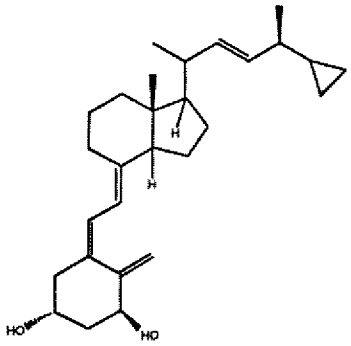
CyDex

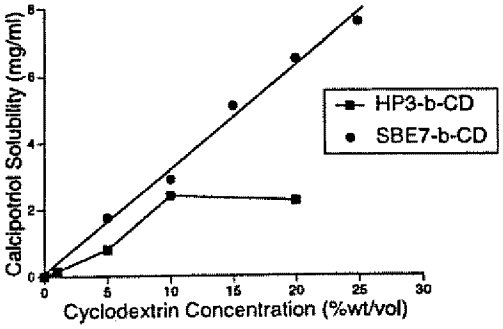
Calcipotriol: Antipsoriatic

~4600-fold Solubility Increase in 20% Captisol

0.0013 mg/ml ⇨7.28 mg/ml

Captisol >3-fold better than HP-β-CD





* T. Loftsson, D. S. Petersen, *Pharmazie*, **52**, 9, 1997

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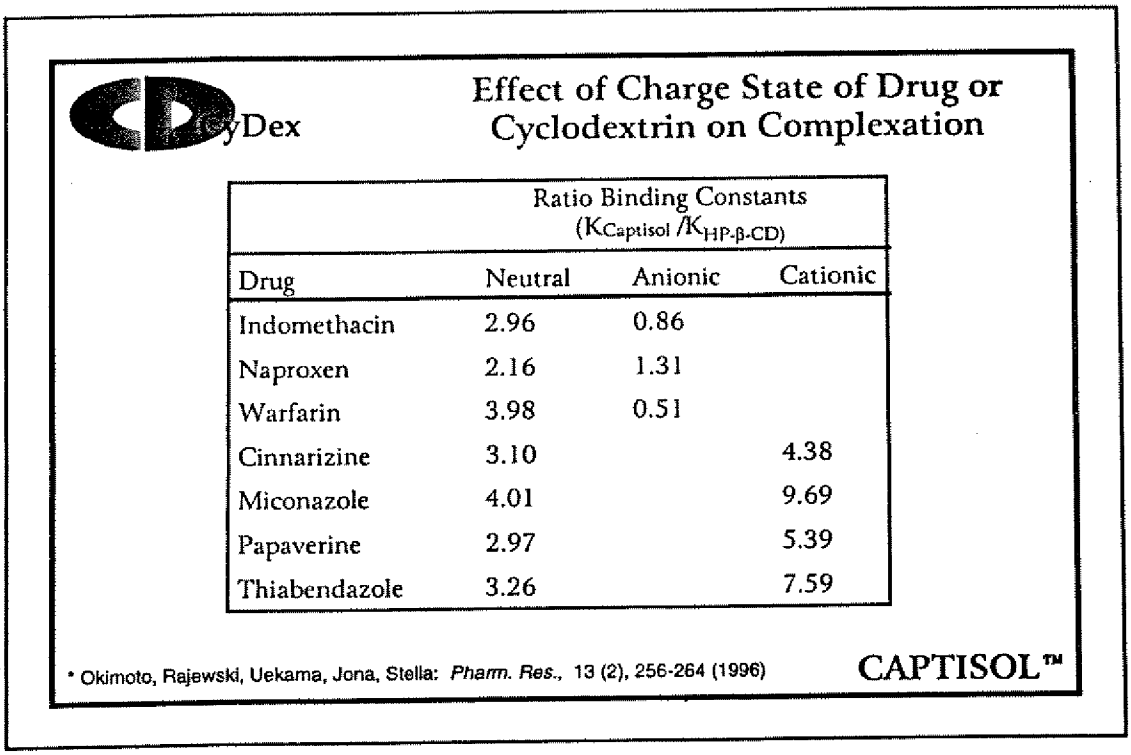
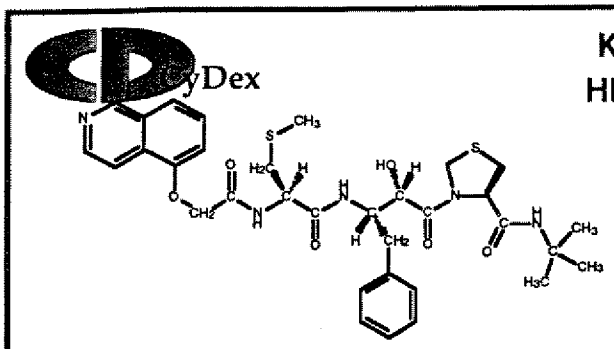


In general, the neutral form of a drug is better at complexing with a CD than a charged drug. The table above shows the ratio of binding constants for a series of drugs that can exist in a neutral or charged state for complexation with the anionic SBE7-β-CD (Captisol) versus with the neutral HP4-β-CD. Clearly, Captisol is 2 to 4 times more effective at complexing the neutral form of the drugs.

Surprisingly, the anionic Captisol molecule is quite capable of complexing with the anionic form of the drugs. For naproxen and indomethacin, Captisol and HP-β-CD are almost equivalent complexing agents. For warfarin, the anionic Captisol is less effective than the neutral HP-β-CD. Evidently the position of the charge in the drug's structure and how this section of the structure interacts with the CD will determine if the charge state of the CD decreases or increases complexation.

For all four cationic drugs, the anionic Captisol is the best CD for complexation. The position of the positive charges in a drug structures must be suitable for complimentary electrostatic attractions to augment the hydrophobic interactions in the CD cavity.



Kynostatin - KNI-272
HIV Protease Inhibitor

Parenteral Formulation

S_o = 4 µg/ml

5mg/ml - pH 3

SBE4-β-CD 15%

Binding Constants (M^{-1}) for Different CD:KNI272 Complexes*

	HP7-β-CD	SBE4-β-CD
$K_{1:1}$	95.1	292.0
$K_{1:2}$	25.5	3.7
$K_{P_{1:1}}$	20.4	96.4
$K_{P_{1:2}}$	3.4	0

* Johnson, Hoesterey, Anderson: *J. Pharm. Sci.*, 83 (8), 1142-1146 (1994)

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Complexation with SBE4-β-CD was utilized to formulate a peptide mimetic, Kynostatin [KNI-272] that functions as an HIV Protease Inhibitor. The intrinsic solubility of KNI-272 is only 4µg/mL and the dose required was 5 mg/mL. This could only be accomplished by using a combination of pH and complexation with SBE4-β-CD.

This example demonstrates again that complexation is more effective with SBE-CDs versus HP-CD. The table above shows that the binding constants for the 1:1 complexation of the unprotonated neutral drug ($K_{1:1}$) are higher for the SBE4-β-CD ($K_{1:1}$ = 292) vs for the HP7-β-CD ($K_{1:1}$ = 95). A similar effect is observed for the protonated drug ($K_{P_{1:1}}$), however the effect is not as dramatic ($K_{P_{1:1}}$ = 96 vs 20) for SBE vs HP-CD.

This study also demonstrated the inability of the anionic SBE-CD to effectively form the 1:2 complex.

Formulation Problem: Proteins

Cyclodextrins & Proteins

Aided Refolding Denatured Proteins

Minimized Aggregation

Improved Isolation of Active Proteins

Stabilized Lyophiles

Provided Improved Activity on Reconsitution

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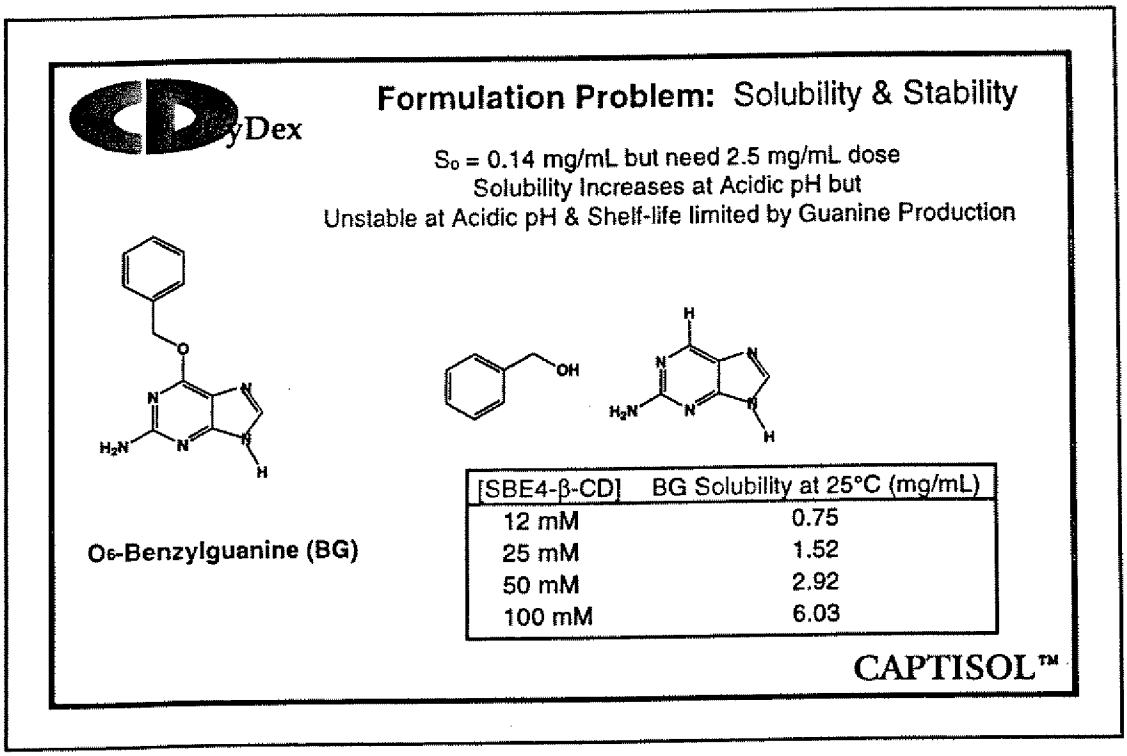
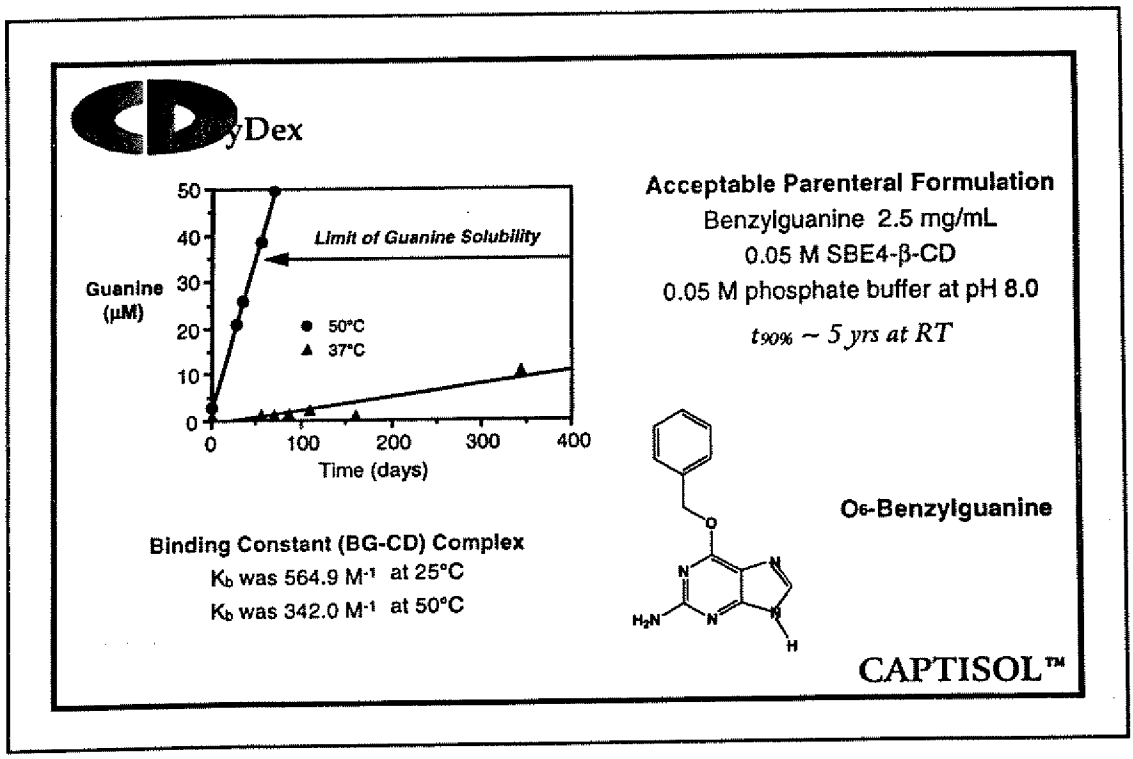


The National Cancer Institute required a parenteral formulation of ^{6}O-Benzylguanine. This required increasing the water solubility ≈20 times its intrinsic solubility of 0.14 mg/mL. One method to increase the solubility was to decrease the pH of the formulation but the benzyl ether group is very susceptible to hydrolysis and in fact the shelf life at neutral pH is limited by the precipitation of guanine.

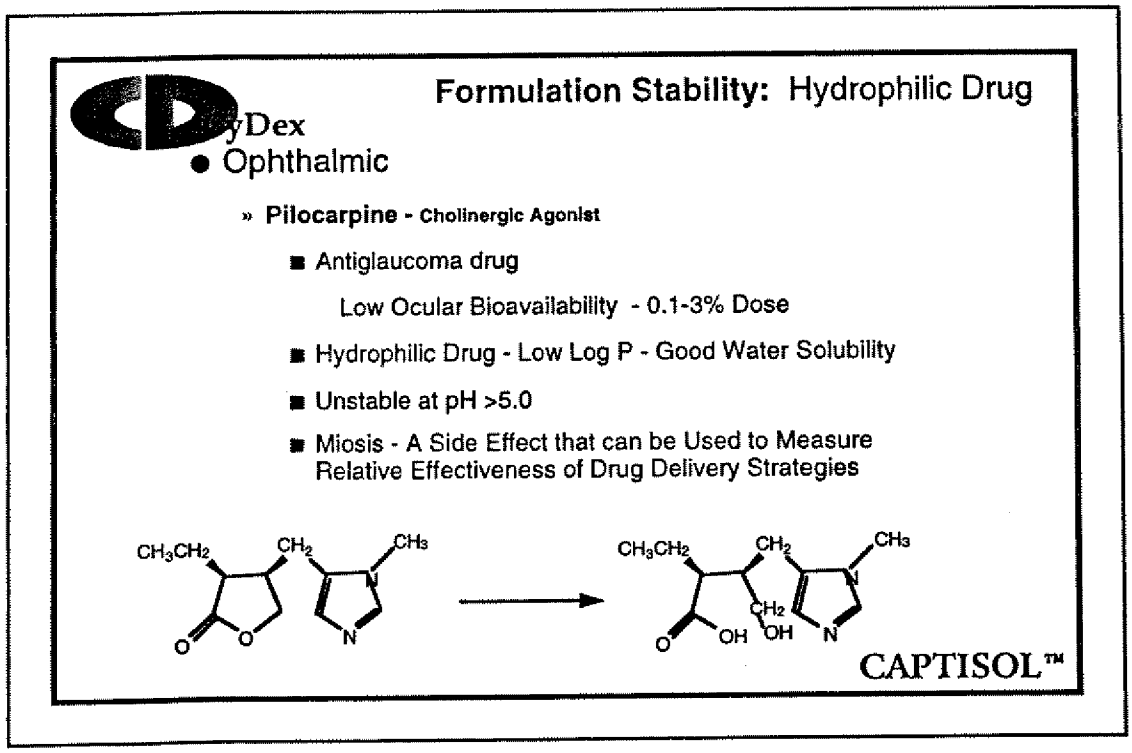
The SBE4-β-CD solutions at 50 mM were able to solubilize the required dose of drug and subsequent accelerated degradation studies on the next slide show that the formulation is quite stable.



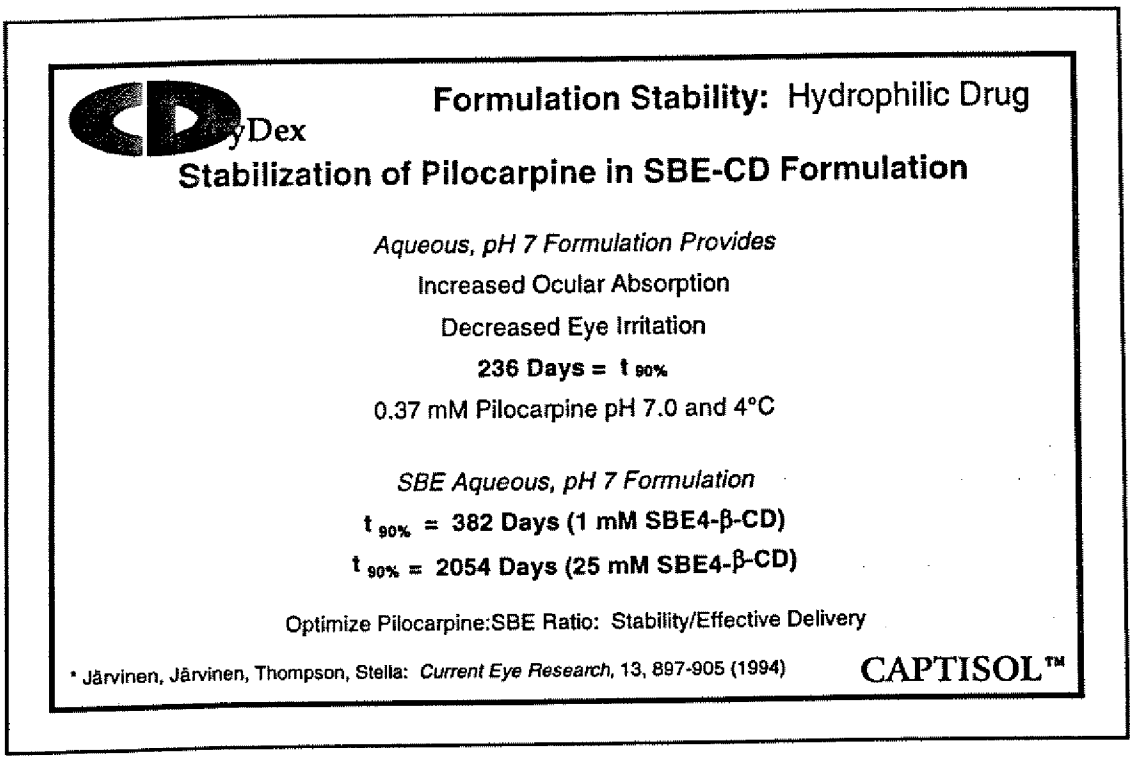
Accelerated degradation studies were conducted on solutions of Benzylguanine:SBE4-β-CD solutions. The stability of the formulation was followed by monitoring the production of guanine. Guanine begins to precipitate at concentrations above ≈35 μM. At 50 °C, the precipitation began in ≈50 days, but at 37°C, extrapolation of the curve indicates that a shelf-life of over 2 years is possible. In fact, the data predict that the $t_{90\%}$ would be ≈ 5 years at room temperature.

These temperature studies show that the binding constants are decreased as the temperature of the system increase. This behavior is understandable from the equilibrium nature of the complexation.

The SBE-CD was able to solubilize and stabilize the formulation of the hydrophobic, benzylguanine.



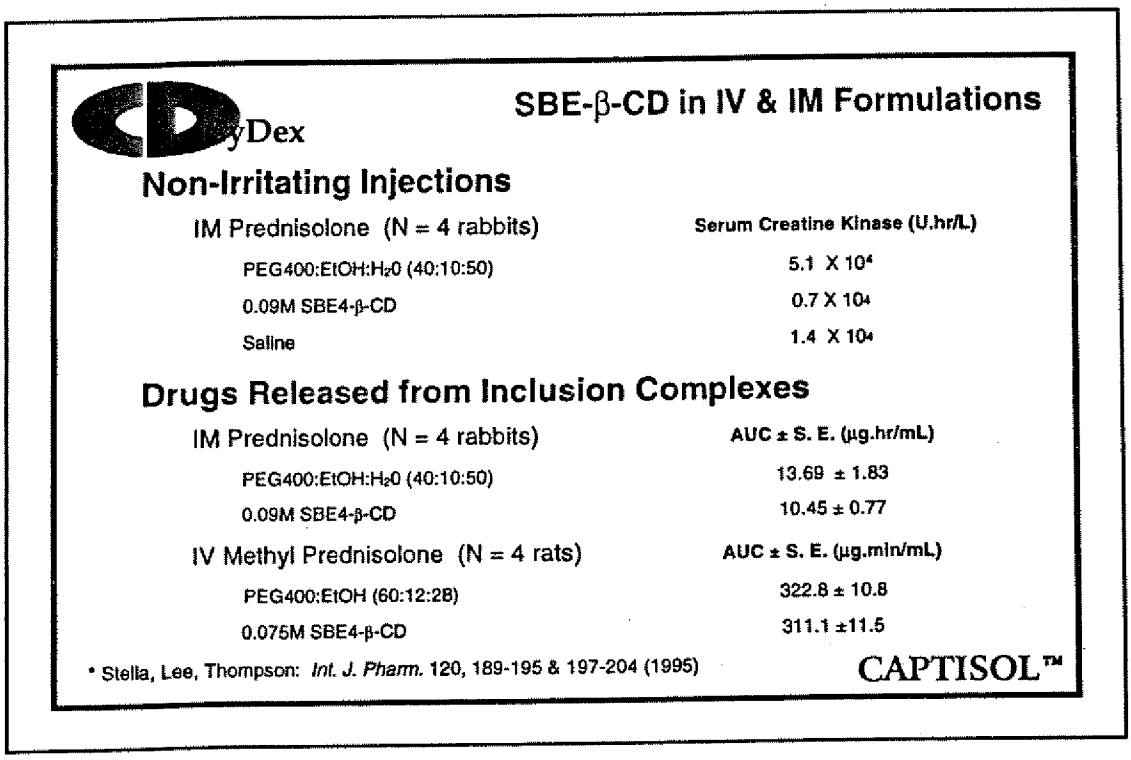
Cyclodextrins are typically considered only when hydrophobic drugs are being studied but even hydrophilic drugs can benefit from complexation. Pilocarpine is a hydrophilic drug with good water solubility but is unstable at physiologically pH. Unfortunately, pilocarpine is more bioavailable and less irritating when formulated at pH 7. Cyclodextrin formulations can be utilized to improve the stability of this hydrophilic drug.



A pH 7 formulation of pilocarpine is less irritating and more bioavailable but the $t_{90\%}$ is only 236 days even at 4°C. Inclusion of a three fold molar excess of SBE4-β-CD to drug increases the stability by 60%. Further increasing the CD concentration to 25 mM provides extensive stabilization ($t_{90\%}$ extrapolated) of pilocarpine.

The appropriate ratio of CD and drug need to optimized for appropriate stability and delivery. Later slides will show that for ophthalmic delivery, too large an excess of CD may limit delivery.

This study shows that even hydrophilic drugs can benefit from complexation with SBE-CDs.



Captisol is being initially developed as a drug carrier system for parenteral formulations. The traditional methods to solubilize hydrophobic drugs for parenteral administration have involved a combination of organic solvents, surfactants and extreme pH conditions. These formulations are often irritating to the patient and may cause adverse reactions.

The data presented above compare two formulation of the steroids, prednisolone and methylprednisolone. One formulation involves the use of cosolvents and the other is an aqueous solution of SBE4-β-CD. In the first study, the muscle damaging effects of the vehicles were evaluated by measuring the release of an enzyme (creatine kinase) into the plasma. Clearly, the cosolvent releases 5 time the amount of this enzyme than the SBE-CD solution which is comparable to a saline injection.

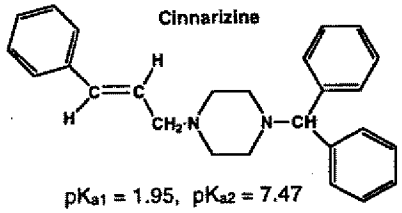
The second study evaluates the amount of drug released in the plasma for and i.m. and i.v. administration of the drugs in cosolvent vs. cyclodextrin formulations. There was no statistical difference between the delivery from either solution. This suggests that CD formulations can be considered in situations where cosolvents have typically be utilized .

SBE-β-CD in Oral Formulations

CyDex

pH	Solubility (mg/100 mL)
2.5	147
4.0	7
5.0	1
6.9	0.07

Cinnarizine



pK_{a1} = 1.95, pK_{a2} = 7.47

Oral Cinnarizine Formulation	F abs (%)*
25 mg - Solid: Capsule	0.8 ± 0.4
25 mg - Aqueous Suspension	8 ± 4
25 mg - 12% w/v SBE4-β-CD Solution	60 ± 13
25 mg - SBE4 Complex: Capsule	38 ± 12

* Beagle Dogs (N = 4)

* Järvinen, Järvinen, Schwarting,Stella: *J. Pharm. Sci.*, 84 (3), 295-299 (1995)

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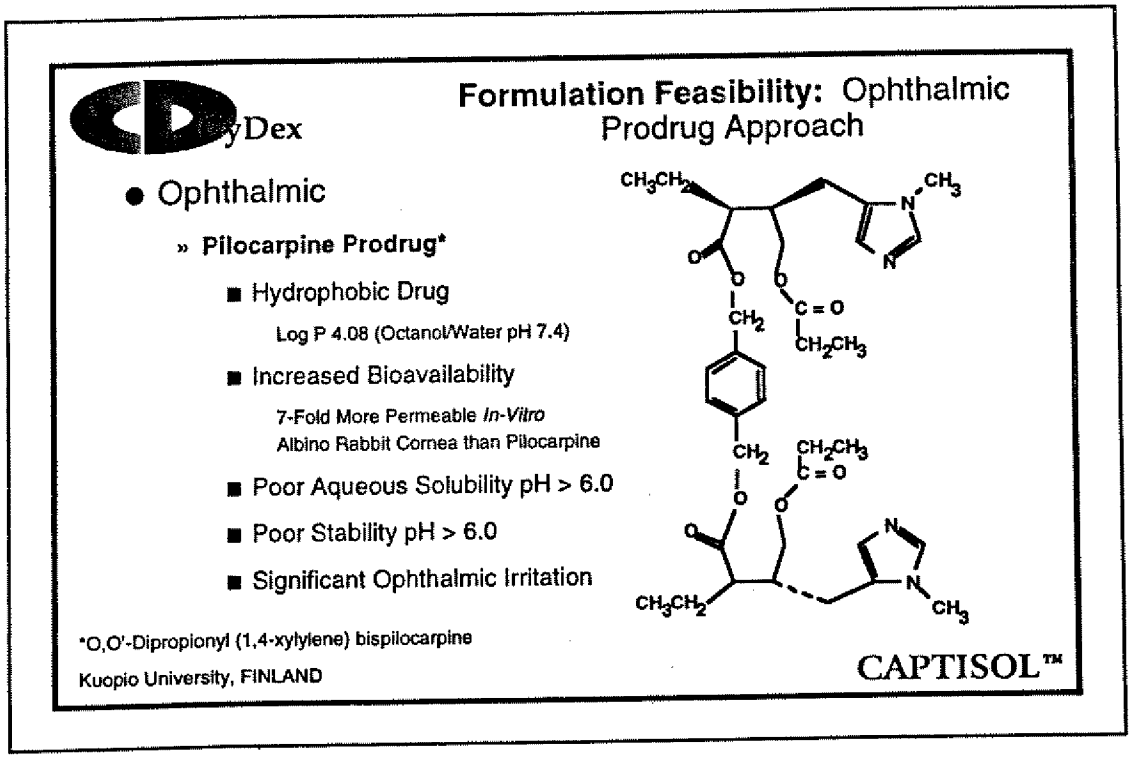
Cyclodextrins can be useful in oral delivery to improve bioavailability. This improvement will be realized if the drugs dissolution rate and solubility are the limiting factors for absorption. Such was the case for cinnarizine.

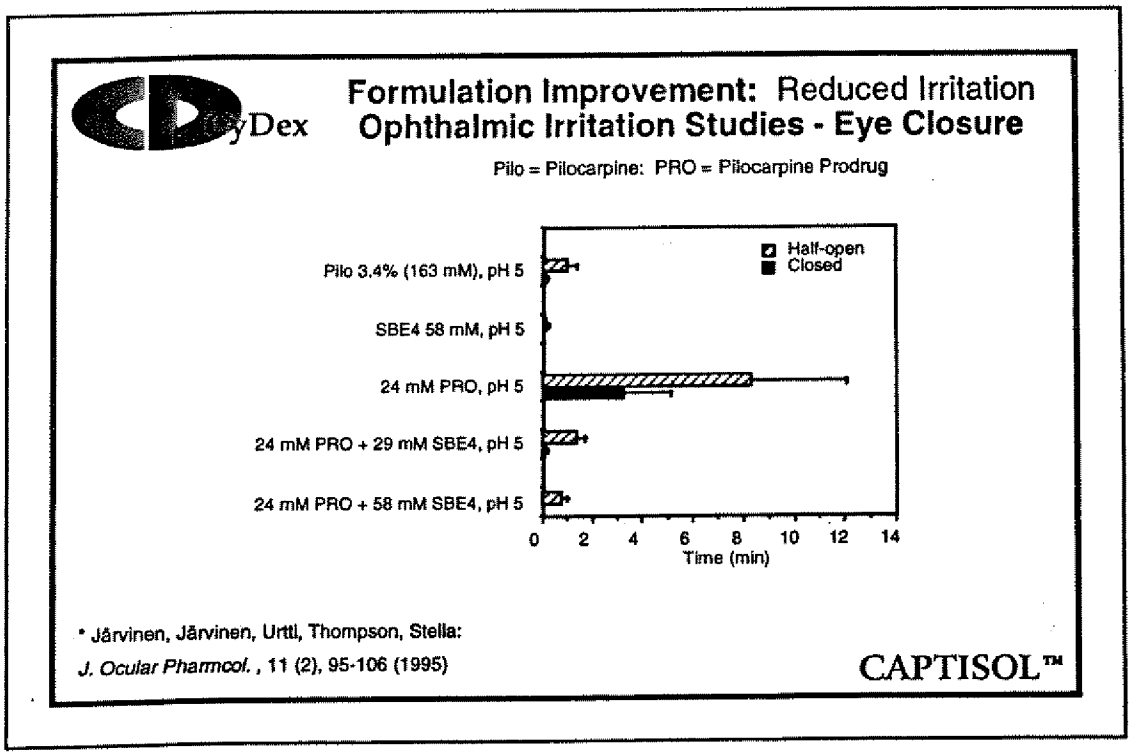
Cinnarizine is used to increase cerebral blood flow particularly with the geriatric patient. Cinnarizine has poor and erratic bioavailability because the absorption depends on the amount of drug that is soluble in the intestines but the drug is very insoluble at the pH of the intestines. The best situation occurs when the drug is readily solubilized in the acid stomach contents and when the contents maintain some acidity as they travel into the upper intestines. Unfortunately, the geriatric patient does not often maintain a pH profile in the GI tract.

The data above show the dramatic improvement in the oral bioavailability of cinnarizine in an SBE4-β-CD solution, or freeze dried preparation packed into a capsule in comparison to the solid cinnarizine or an aqueous suspension.



Cyclodextrin formulations can be utilized to minimize irritation effects of very hydrophobic active agents. Researchers at Kuopio University (Finland) have patented a prodrug of pilocarpine. The prodrug was designed to increase the delivery of pilocarpine by increasing the hydrophobicity of the drug and thus increasing its bioavailability. This was achieved but the resulting compound now is not very water soluble, is still unstable at physiological pH and is very irritating to ocular tissue.

The SBE-CDs are able to solubilize the prodrug and from previous slides it should be clear that they can also stabilize the formulation.

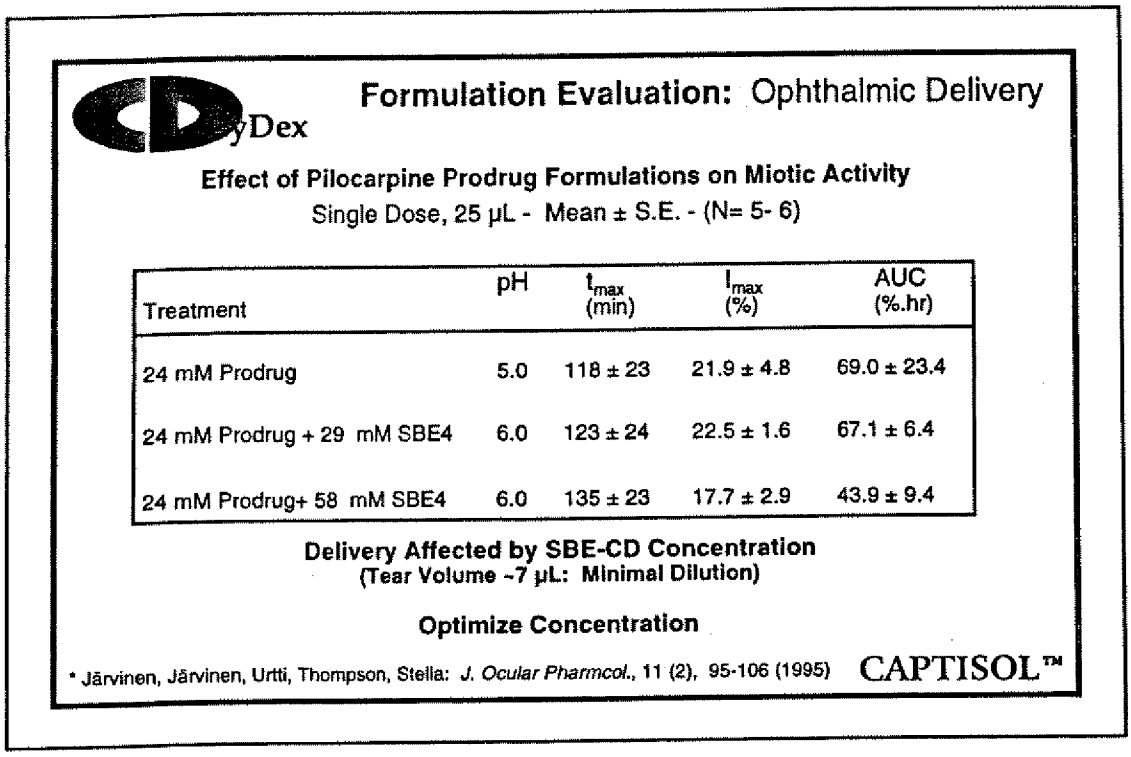


The irritating effect of ophthalmic formulations can be determined by measuring the time rabbits close their eyes following instillation of a formulation. The bar graphs above show the effect of a commercial pilocarpine formulation as the standard to compare the prodrug (PRO = pilocarpine prodrug) formulations. The SBE4 vehicle is very well tolerated by the rabbits - much like a saline wash.

The third set of data show the irritating effect of the prodrug at a concentration that can be dissolved in water without any additives. [Note: the 24 mM concentration of the prodrug elicits the same pharmacological activity as the 163 mM dose of pilocarpine - showing the improved bioavailability of the prodrug]

Incorporation of an equal molar quantity of SBE4-β-CD remarkably decreases the irritation of the prodrug. Further increasing the SBE4-β-CD concentration further reduces the irritation.

Therefore, it is possible to solubilize, stabilize and reduce the irritating effects of the pilocarpine prodrug using an SBE-CD formulation. However, the amount of SBE-CD used in the formulation must be optimized in order not to affect the delivery of the active *in-vivo*.

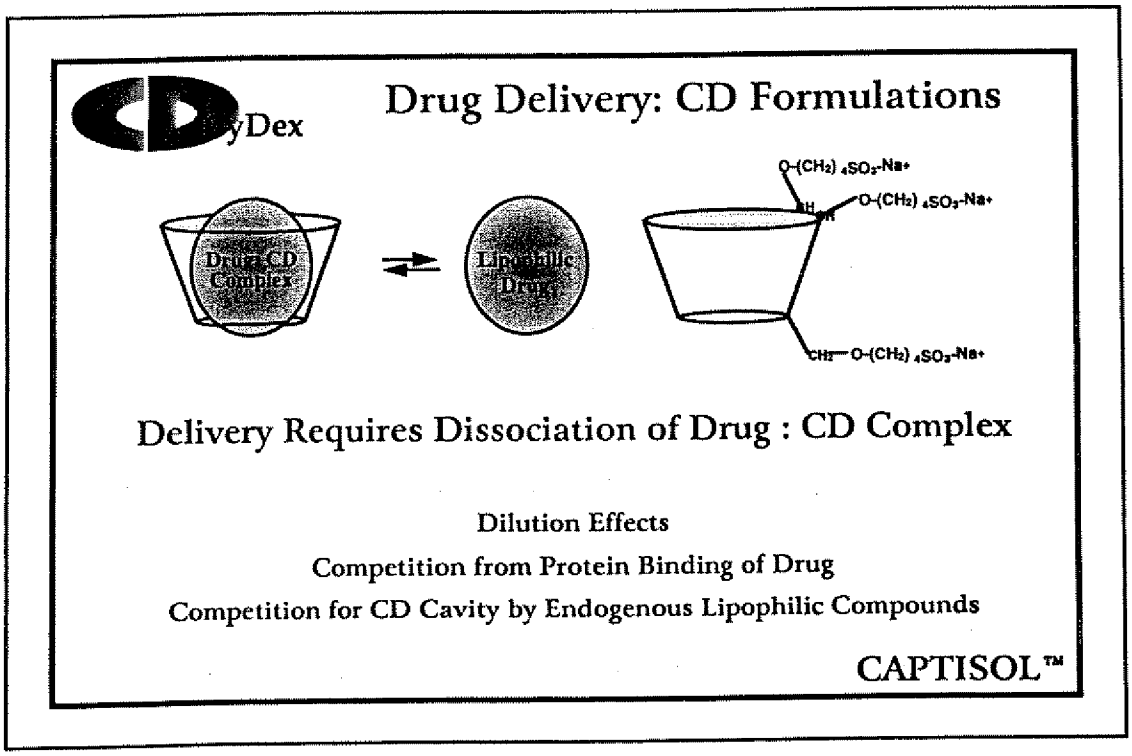


The amount of SBE-CDs used in any formulation must be optimized in order not to affect the delivery of the active *in-vivo*. This caution is particularly important for formulations used for ophthalmic, nasal and topical delivery because there is minimal dilution to promote dissociation of the drug.

The delivery of the pilocarpine prodrug was studied not by measuring drug plasma concentrations but by measuring a pharmacological side effect of the drug, the miotic effect. Miosis is the enlargement of the diameter of the pupil and can be quantitated through the use of photographs of the rabbit eyes.

The data above show that the delivery of the prodrug from the aqueous formulation and the 1:1 molar formulation with SBE4-CD are equivalent. However, increasing the SBE concentration to a 1:2 molar excess results in a decrease in the effective delivery of the prodrug as observed with the reduction of the AUC from 69 to 44 %.hr.

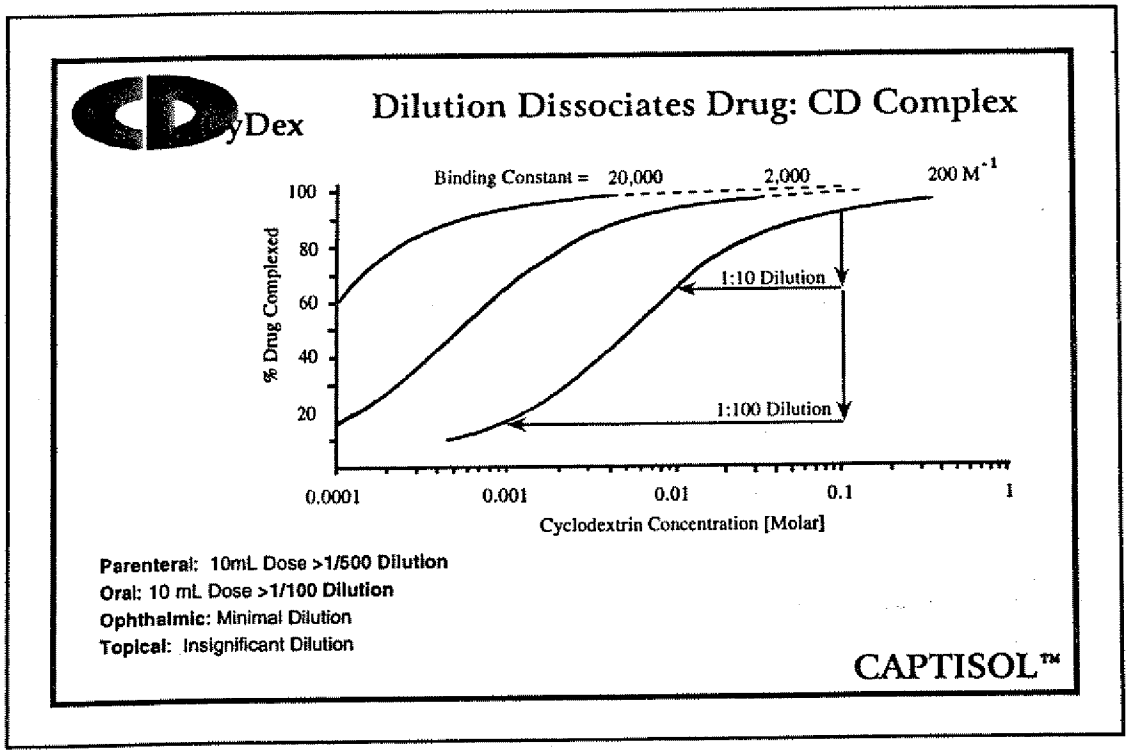
The optimal amount of SBE-CD to use in each formulation will vary with the drug but needs to be optimized for all of the effects desired, solubility, stability, reduction of side effects and delivery.



Drugs complexed to cyclodextrins must dissociate in order to exert their pharmacological effect. Dissociation of the complex occurs readily upon dilution in the blood stream or in the GI tract. Dilution effects will be less operative when the routes of administration are ophthalmic, nasal or topical. Under this routes of administration, the concentration of excess CD in the formulation must be carefully optimized so as not to adversely limit delivery.

In addition to dilution effects promoting the dissociation, drug may also be 'pulled' from the cavity for complexation to proteins circulating in the blood system. When considering the amount of protein in the blood stream vs an i.v. dose of Captisol, the hydrophobic drug has a lot more protein to bind to than Captisol!

Other endogenous lipophilic compounds (cholesterol, bile salts, etc) can also compete for the CD cavity and 'push' the drug from the complex.

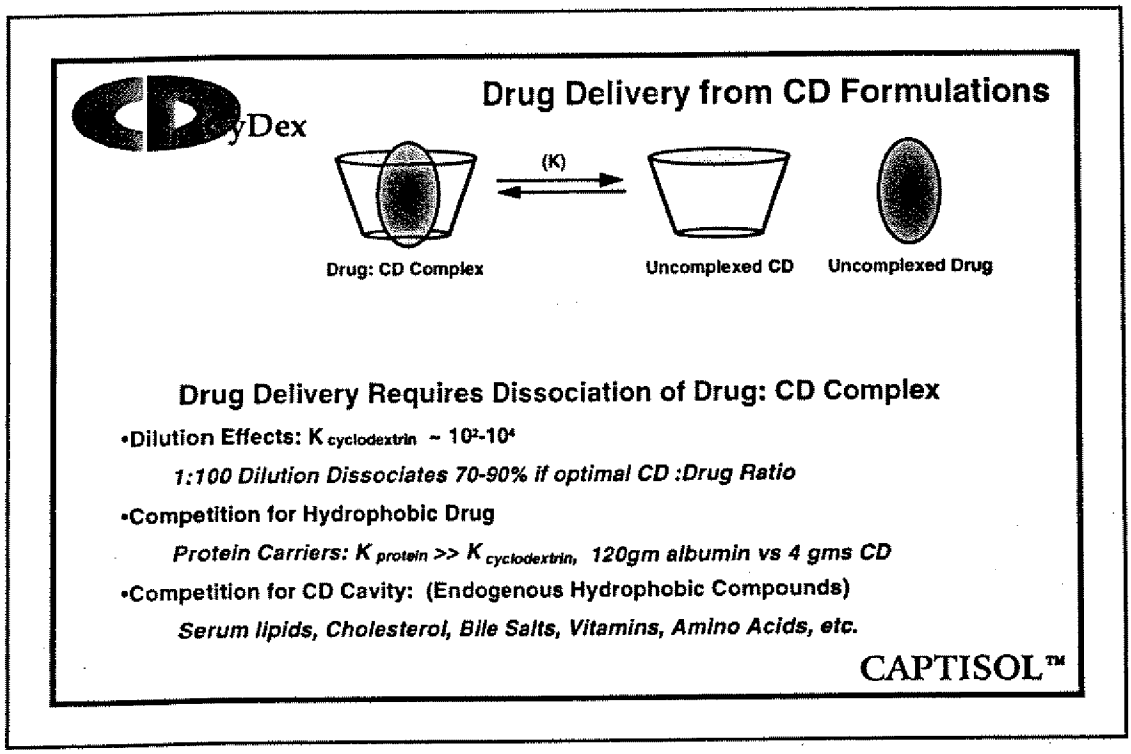


Dilution Dissociates the Drug:CD Complex

For a drug with a binding constant of 200 M^{-1} and formulated at 0.1M CD solution, a 1:10 dilution with result in only ~65% of the dur remaining complexed and ~35% of the drug exisiting in the free form. Another 1:10 dilution (total 1:100 dilution) results in further dissociation and the only ~20% of the drug remains complexed and 80% of the drug is in the free state.

There is sufficient dilution on parenteral and oral administration to result in almost complete dissociation of the drug:CD complex. This however is not the state for opthalmic or topical delivery where dilution effects are minimal.

For any route of administration the drug:CD ratio should be optimized. An excess of CD for any formulation can limit delivery. For example a drug with a binding constant of 20,000 M^{-1} should need only a 0.001M CD solution to be fully complexed but if this drug is formulated in 0.1M CD solution, then a 1:100 dilution will only bring the CD concentration down to that which will keep all of the drug complexed! Therefore, formulators need to sure they are using only the amount of CD needed otherwise the delivery of the drug in-vivo may be adversely affect due to excess. CD.



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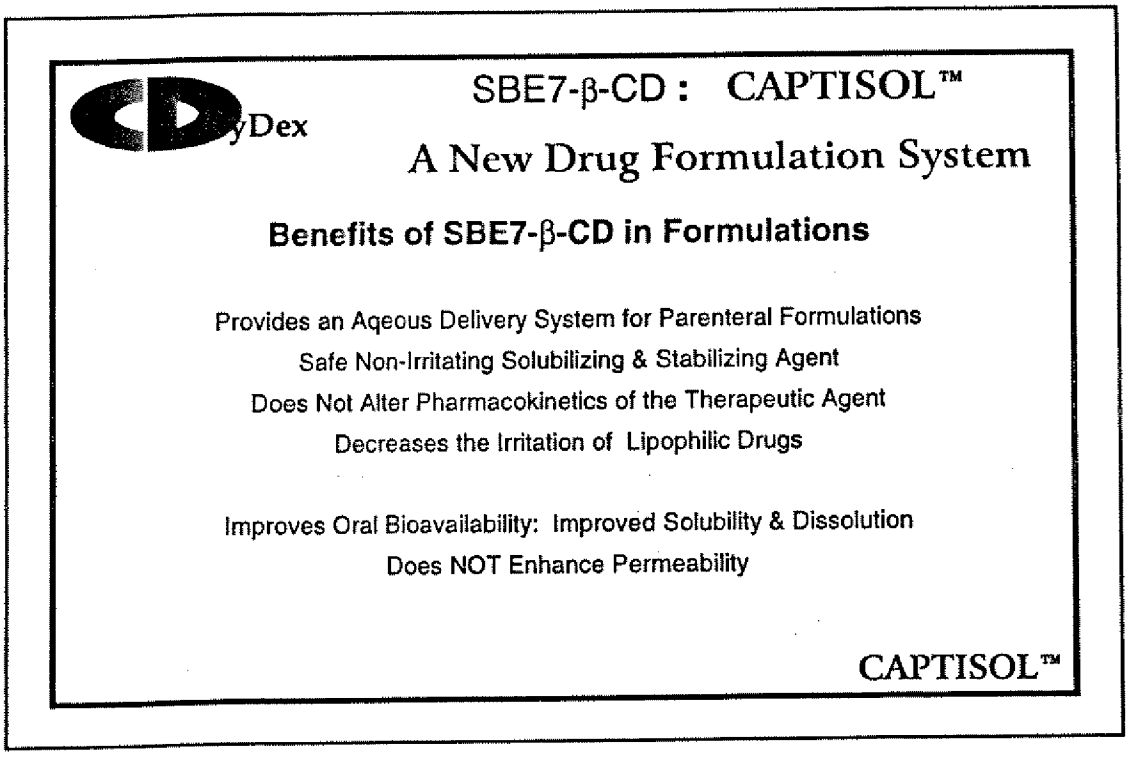
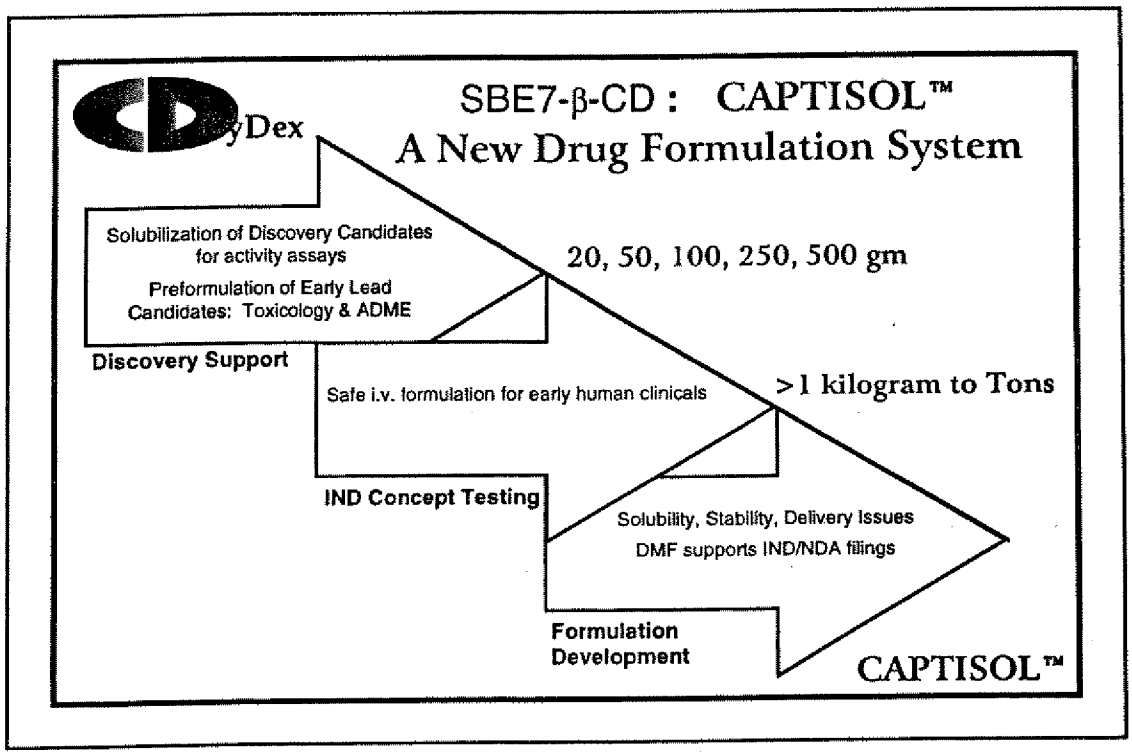


Captisol is a drug carrier system that can solubilize, stabilize and deliver hydrophobic and hydrophilic drugs by multiple routes of administration.

Captisol is biocompatible, safe and devoid of any pharmacological activity. Captisol formulations can be used to decrease the irritation of active ingredients.

Captisol formulations exhibit delivery characteristics comparable to cosolvent formulations without the use of organic solvents and harsh surfactants.



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